High-performance liquid chromatography was conducted on a Du Pont Model 8800 instrument (USA) using a Zorbax ODS column (0.46 × 25 cm). The proteins were detected by means of UV spectrophotometer with a flow-through cell at a wavelength of 220 nm. The protein fractions were selected with the aid of a ISCO Model 1220 fraction collector (USA) and were freeze-dried. The column was thermostated with an accuracy of ±0.2°C.

Amino acid analysis was carried out after the hydrolysis of the fractions with 6 N HCl (105°C, 24 h) on a Hitachi 835 amino acid analyzer (Japan). The working concentration of the sturins was 5 mg/ml, and the volume of sample introduced 10-20 μl.

The optimization program was drawn up in BASIC language and the volume of memory occupied was 3.5 kilobytes.

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AMINO ACID COMPOSITIONS OF PROTEINS SYNTHESIZED IN VITRO IN THE CELL NUCLEI OF EUKARYOTES

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The proteins synthesized in vitro by the nuclei of animal and plant cells differ in their amino acid compositions. The differences found show the species specificity of the products.

It has been shown previously (from the inclusion of [^{14}C]amino acids) that animal and plant cell nuclei synthesize proteins under different conditions [1, 5]. The products synthesized by the nuclei of neurons of animal brains and of plants (the cotton plant) coincide in molecular mass. It appeared of interest to compare their amino acid compositions.

Table 1 gives the results obtained in a determination of the amino acid compositions of the nuclear proteins.

A species specificity of the amino acid compositions of the proteins synthesized by the nuclei of rabbit and bovine brain neurons was found. We have established that in both proteins synthesized by brain neuron nuclei there are larger amounts of such amino acids as lysine, arginine, glutamic acid, proline, alanine, and valine in the bovine nuclear proteins with the amounts of histidine, aspartic acid, threonine, serine, glycine, and tyrosine 2-12 times higher than in the rabbit proteins.

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TABLE 1

Amino acid	Nuclear protein of brain neurons synthesized in vitro		Proteins synthesized in vitro in the nuclei of cotton seed shoots
	rabbit	bovine	
1. Lysine	9 (8,8)	16 (16,1)	4 (4,1)
2. Histidine	2 (2,1)	10 (10,7)	4 (4,3)
3. Arginine	9 (9,0)	22 (21,4)	20 (19,8)
4. Aspartic acid	3 (3,4)	18 (17,8)	32 (31,6)
5. Threonine	2 (2,0)	14 (14,2)	12 (11,8)
6. Serine	2 (1,7)	24 (24,1)	16 (15,9)
7. Glutamic acid	14 (13,6)	29 (24,4)	28 (28,1)
8. Proline	9 (8,9)	16 (16,0)	8 (8,1)
9. Glycine	2 (2,3)	13 (13,4)	8 (7,9)
10. Alanine	13 (13,2)	19 (18,8)	12 (11,6)
11. Valine	8 (8,1)	11 (11,6)	8 (7,9)
12. Isoleucine	5 (4,9)	8 (8,0)	12 (11,6)
13. Leucine	7 (6,6)	15 (15,2)	8 (7,9)
14. Tyrosine	2 (1,8)	14 (14,3)	2 (1,7)
15. Phenylalanine	4 (3,8)	10 (9,8)	4 (4,1)
16. 1/2-Cystine	7 (7,2)	—	—
17. Methionine	Tr.	5 (4,7)	4 (3,7)

Cotton-plant cell nuclei synthesize proteins containing arginine, aspartic acid, threonine, serine, glutamic acid, alanine, and isoleucine. The amounts of arginine and glutamic acids are almost equivalent in the proteins synthesized by bovine nuclei and cotton-plant nuclei. The amount of tyrosine in the cotton-plant protein is smaller, as also in the rabbit protein. No half-cystine was detected either in the bovine protein or in the cotton-plant protein.

The results obtained demonstrate a substantial species specificity and, as has been shown previously [6, 7], tissue specificity of the nuclear synthesis of proteins which is shown in their composition and, consequently, in their structure. It is important that the proteins synthesized by brain (bovine) neuron nuclei contain no carbohydrate residues.

EXPERIMENTAL

The work was performed on neurons from the brains of rabbits from one litter of 6-8 individuals and from bovine brain. Three-day shoots of seeds of a cotton plant of the Tashkent-8 variety were used in parallel.

The neuronal cells of the cerebral cortex were carefully separated from the glial cells by microdissection, and the neuronal cells were comminuted by hand in a homogenizer with solution A (in a ratio of 6 ml/g of tissue), which contained 0.32 M sucrose, 0.003 M $MgCl_2$, and 0.001 M K_2HPO_4, pH 7.4. A suspension of nuclei with cell fragments was obtained. The suspension was centrifuged at 3000 rpm for 15 min. The supernatant liquid was sucked off, and the deposit of nuclei in the bottom of the tube was suspended in a small volume (1.5-2.0 ml) of solution A and was transferred into a test-tube with a previously prepared density gradient of 1.8, 2.0, 2.2, 2.4, and 2.6 M sucrose. The homogenate of nuclei was gently placed on the top layer and centrifugation was carried out on a Beckman continuous ultracentrifuge (USA) with a SW-27 rotor at 78,000g for 60 min. After centrifugation, the cytoplasmic residues had collected in the form of a film at the surface, and they were removed very carefully, while the neuronal nuclei that had gathered between the 2.2-M and the 2.4-M sucrose were taken off with a syringe. This suspension of nuclei was resuspended in a large volume of the initial solution A and the suspension was centrifuged at 3000 rpm for 10 min. This procedure was repeated twice in order to wash them free from sucrose. The neuronal nuclei formed a colorless deposit. The deposit was suspended in 0.25 M sodium phosphate buffer solution with pH 7.4. Drops were taken from the suspension to check purity and integrity in an optical microscope. The nuclei isolated were used for the in vitro synthesis of protein.

The synthesis of protein in the isolated nuclei of brain neurons and cotton shoots was performed under the corresponding conditions. The extraction of the nuclear fractions was carried out under strictly standard conditions.

The labeled proteins after the incubation of the nuclei were extracted from the TCA-insoluble residues into 0.025 M Tris + 0.192 M glycine buffer, and then extraction was con-

tinued into 6 M urea + 0.1% Na dodecylsulfate and 0.14 M β-mercaptoethanol. The desalted and freeze-dried combined protein extracts were subjected to gel filtration on a column of Sephadex G-50 (fine) (Pharmacia, Sweden) equilibrated with 0.05 M ammonium acetate buffer, pH 6.06. The column had dimensions of 1.1 × 102 cm and a volume of 100 ml. The rate of elution was 20 ml/h.

All the fractions were collected and dried, and on subsequent separation in a high-performance liquid chromatograph two radioactive peaks (other peaks were not radioactive) of electrophoretically homogeneous proteins with molecular masses of approximately 25-30 kDa (I) and 10-15 kDa (II) were isolated. The proteins (I) isolated from the nuclei of rabbit and bovine neuron cells and also from those of the cotton plant were lyophilized and used to determine their amino acid compositions. The proteins were hydrolyzed as described in [8]. Amino acid compositions were determined on a Biotronik IC-7000 analyzer (FRG).

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CHLORINATION OF LIGNIN AT LOW TEMPERATURES

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A high reactivity of the olefinic double bonds of the lignin (Lg) macromolecule in the reaction with molecular chlorine has been found which has permitted the chlorination of Lg to be carried out under cryogenic conditions at 150-250 K. The process takes place by a radical-chain mechanism with a length of the kinetic chain of approximately 1000 units. The bulk of the chlorine is consumed in the olefinic chains of the Lg. Other reaction products besides chlorolignin are hydrogen chloride and phenoxyl radicals, which have singlet ESR spectrum (ΔH = 1.0-1.2 mT) and are retained in the chlorinated samples of Lg to ~250 K.

The reaction of chlorine with lignin (Lg) presents considerable interest for the study of the mechanism of the bleaching process that is widely used in the pulp and paper industry. Lg chlorination processes are usually considered at temperatures above 270 K. Under these conditions ionic reactions of chlorine with the aromatic ring [1] are usually discussed; a free-radical mechanism has been regarded as unlikely. However, Lg is also a polyolefin with a high content of double bonds in the side chains, and therefore it is possible to expect the appearance of features characteristic for the radical-chain chlorination of olefins.

In order to separate the different stages of the process and to reveal the relative reactivities of the functional groups of the macromolecule, in the present work we have investigated the low-temperature (150-250 K) chlorination of hydrolysis lignin by molecular chlorine.

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