PROTEINS SYNTHESIZED IN VITRO IN THE CELL NUCLEI OF VARIOUS RABBIT ORGANS

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UDC 612:591.04

The differences in the composition of the proteins that have been detected show a tissue specificity of the products synthesized by the nuclei of these cells.

In spite of the fact that the nuclear polypeptide synthesis of proteins was discovered as long ago as 1955 [1], this phenomenon has come to be regarded as a solidly established fact for eukaryote nuclei only recently [2, 8]. However, the functional value of this process is still unknown. In particular, the protein formed de novo in cell nuclei has been studied extremely inadequately.

Zimmerman et al. [3], studying proteins in the nuclei of Hela cells from the de novo inclusion of labeled amino acids, detected two radioactive peaks one of which corresponded to histones and the other to an unknown protein. Laval and Mouli [4] isolated proteins labeled with [^{14}C] amino acids synthesized in vitro by the nucleus-nucleolus fraction. Radioactivity was not distributed along the whole polypeptide chain but both in the center and in two or three points at the C-end. K. S. Matinyan and S. R. Umanskii [5], using rat liver chromatin as template showed the possibility of the in vitro synthesis of polypeptides with molecular weights of ~6 kD.

Thus, it has been shown experimentally that proteins are synthesized in nuclei of various origins, but their nature, organ specificity, and functions remain unelucidated.

In the present paper we consider an attempt to determine the difference in the composition of proteins synthesized de novo by the cells of various animal organs.

We had previously isolated two protein fractions with molecular weights of approximately 10-15 and 25-30 kD synthesized de novo from nuclei of rabbit-brain cells (neurons). In parallel, we investigated the protein fractions from liver and thymus cell nuclei. It was found that in the nuclei of brain neurons and the nuclei of liver and thymus cells the protein fractions synthesized de novo are glycoproteins [6, 7]. In particular, it was established by TLC that the carbohydrate moiety of the protein fraction with a molecular weight of 10-15 kD included the neutral monosaccharides xylose and glucose, the xylose making up the greater part of the monosaccharides.

In the present communication we give the results of an investigation of the amino acids composition and the qualitative and quantitative contents of neutral monosaccharides in the proteins/glycoproteins with a molecular weight of 25-30 kD isolated from nuclei of brain neurons and of liver and thymus cells.

The monosaccharides (in percentages of the total amount of proteins) were:

	Brain (neurons)	Liver	Thymus
Xyl	11.70		2.47
Man	3.17	0.46	0.10
Glc	2.33	3.24	0.11
Gal	1.38	2.48	1.15
Galacturonic acid		3.59	

As we can see, the amount of galactose was almost the same for all three proteins, but there were substantial differences in the amounts of the other sugars. Thus, there was

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Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 565-568, July-August, 1987. Original article submitted January 23, 1987.

0009-3130/87/2304-0473$12.50

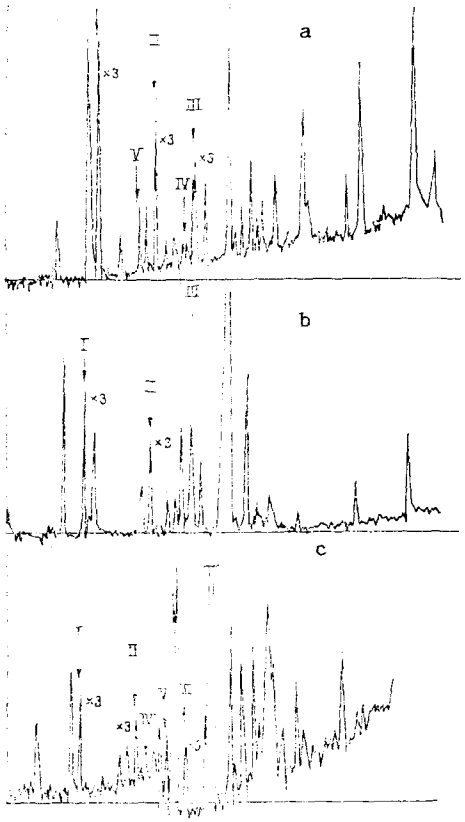

Fig. 1. Monosaccharide composition of the proteins synthesized in vitro by the cell nuclei of brain neurons (a), of liver (b), and of thymus (c): I) xylose; II) mannose; III) glucose; IV) galactose; V) galacturonic acid; (results obtained by the GLC method).

almost five times as much xylose in the neuron proteins as in the thymus proteins, and xylose was completely absent from the liver proteins; mannose was present in all three of the proteins studied but its amount was 6-30 times greater in the brain proteins. The amount of glucose in the thymus proteins was insignificant, and only the proteins isolated from liver cell nuclei contained galacturonic acid.

Thus, the proteins synthesized in vitro by the cell nuclei of various organic differed with respect to the amounts of neutral monosaccharides that they contained. A determination of the amino acid compositions of the glycoproteins gave the following results:

	Brain (neurons)	Liver	Thymus
Asp	5.6	7.0	10.9
Thr	2.6	5.1	6.4
Ser	5.2	5.8	7.2
Glu	8.1	10.8	15.5
Pro	3.7	4.2	5.9
Gly	8.9	17.3	21.7
Ala	4.5	9.1	10.7
1/2 Cys	0.17	1.1	1.6
Val	2.4	5.4	6.7
Met	1.1	1.9	0.3
Ile	1.6	4.1	5.1
Leu	3.4	7.7	10.3
Thyr	1.6	3.1	3.3
Phe	1.7	2.5	4.0
His	1.0	3.4	4.8
Lis	3.5	9.4	11.4
Arg	3.0	9.4	3.3

Tryptophan was not determined.

As we can see, the amounts of aspartic acid and serine were greater in the brain neuron nuclei. The amounts of glutamic acid, glycine and aspartic acid residues in the proteins of the thymus cells were almost 1.5-2.0 times greater than their amounts in the proteins synthesized by the neuron and liver nuclei, while the amounts of phenylalanine, serine, and proline residues were approximately the same.

Thus, proteins synthesized in vitro by the cell nuclei of various organs differ in their amino acid composition.

EXPERIMENTAL

Three or four animals from a single litter were used.

The nuclei from the cells of the brain, the liver, and the thymus were isolated by a known method [8]. Protein synthesis in the isolated nuclei was performed under the same conditions [9]. The extraction of the labeled protein from the nuclei, their fractionation on Sephadex G-50, and hydrolysis were carried out as described previously [6, 8, 9]. The amino acid compositions of the protein fractions were determined on a Biotronik IC-7000 analyzer (FRG) and the amounts of neutral monosaccharides were determined by the GLC method on a Hewlett-Packard 5710 A chromatograph (USA) with a flame-ionization detector. The carrier gas was helium and the pressure at the inlet 1.8 atm. Temperature conditions: 100°C for 4 minutes and then a rise in temperature at the rate of 4°C per minute to 220°C; 0.24 mm × 40 m capillary column with the phase SE-30. The methylation of a sample (200 μg of glycosides) was performed in 1.5 N HCl in absolute methanol under argon in sealed tubes at 100°C for 24 h. After this, two equivalents of pyridine + acetic anhydride were added (200 μl of methanolysis mixture + 30 μl of pyridine + 40 μl of acetic anhydride) and the mixture was evaporated. Then the trifluoroacetyl derivatives of the methyl glycosides were obtained as described by Bryn and Jantzen [10].

CONCLUSION

The protein synthesized in vitro by the nuclei of cells of various organs of the rabbit differed in their contents of neutral monosaccharides and in their amino acid compositions. The differences found show a tissue specificity of the products synthesized by the nuclei of the cells

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