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ACTION OF THE PRODUCTS OF THE DEGLYCOSYLATION OF THE NUCLEAR GLYCOPROTEIN OF RABBIT BRAIN NEURONS ON THE POLYMERASE ACTIVITY OF THE NEURONAL NUCLEI

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The influence of a glycoprotein and its fragments on the RNA polymerase activity of nuclei has been studied. Both the glycoprotein itself and its fragments suppress the RNA polymerase activity of the system in vitro, *and the carbohydrate moiety suppresses the activity more strongly than the protein moiety.*

In recent years reports have appeared in the literature showing the existence of low-molecular-mass substances of peptide nature in the cytoplasm of the cells of various organs of animals [1, 7]. It has been established that some of them regulate the activity of enzymes and permeability of membranes in mitochondrial protein synthesis. However, the action of low-molecular-mass polypeptides synthesized in the cell nucleus (especially glycoproteins and their fragments) on the process of transcription in the nucleus has not been studied.

It has been shown previously that glycoproteins (GPs) synthesized in the nuclei of rabbit brain cells suppress the activity of RNA polymerase [6]. Continuing these investigations, we have attempted to find whether individual deglycosylated fragments of the rabbit nuclear glycoprotein (NGP) possess the same activity.

We first carried out an amino acid analysis and determined the composition of the carbohydrate component (in nmoles):

Lys	8.76	Gly	13.67
His	2.10	Ala	13.23
Arg	9.03	Val	8.15
Asp	3.42	Ile	4.91
Thr	2.02	Leu	6.60
Ser	1.75	Tyr	1.79
Glu	2.28	Phe	3.77
Pro	8.84	1/2Cys	7.27
		Met	Tr.

The composition of the NGP lacked methionine but included large amounts of lysine, arginine, proline, glutamic acid, alanine, and valine. The tryptophan content was not determined, since this amino acid is completely destroyed in the process of acid hydrolysis.

For the qualitative determination of the carbohydrate composition we used thin-layer and gas–liquid chromatographies (TLC and GLC). In the thin-layer separation of the sugars on silica gel, for the brain GP we used the butanol–acetone–water (4:5:1) system, and on the starting line we deposited glucose and mannose as markers and, beside them, the NGP hydrolysate. In this hydrolysate we detected xylose, mannose, and glucose (Fig. 1). The xylose gave a darker-colored spot; i.e., its content was more than 3 times that of glucose and mannose.

Some sugars, present in small amount, were not detected by the TLC method, and, therefore, for a more detailed investigation of the levels of xylose, mannose, and glucose in the GP we used GLC. The results showed (Table 1) that the main components in the sugar moiety were xylose, mannose, and glucose, while galactose and galacturonic acid were present in very small amount (Fig. 2).

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TABLE 1. Composition of the Monosaccharides of the GP Synthesized by the Nuclei of Brain Neurons

Monosaccharide	Carbohydrate components of the GP, wt.%
Xylose	11.7
Mannose	3.17
Galactose	1.38
Glucose	2.33
Galacturonic acid	Tr..

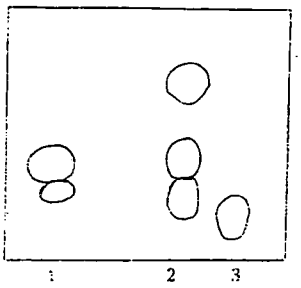

Fig. 1. Monosaccharide composition of the GP synthesized in the neuronal nuclei (determined by the TLC method). Solvent: butan-1-ol-acetone–water; revealing agent: aniline phthalate: *1*) mixture of mannose and glucose; *2*) glycoprotein hydrolysate; *3*) glucose.

Attention must be directed to the fact that, according to the literature, xylose is rarely found as a component of protein. Thus, the GP of human blood serum has been shown to include glucose, fructose, mannose, galactose, and sialic acid, with only a small amount of xylose [10]. In the NGP, the amount of xylose was greater than that of the other sugars.

Thus, xylose, glucose, and mannose are the main structural components of the carbohydrate part of the NGP molecule. The results of these investigations were confirmed by NMR spectroscopy. The sialic acid content was 2 μg in a 50-μg sample. Deglycosylated fragments are used mainly for determining the structure of the glyco part of a GP.

We have determined the action of individual fragments of the carbohydrate part of the GP on RNA polymerase activity with the aim of establishing the composition of the main functioning part – i.e., whether this is the protein fragment or the carbohydrate fragment.

As can be seen from Fig. 3, on the addition to the nuclei of the protein part (*1*) of the GP in a dose of 5 μg per sample, the activity of the RNA polymerase fell by 41%. When the dose was increased to 7.5 and 10.0 μg, the activity of the enzyme fell by 36 and 41%, respectively. Consequently, in these doses the protein part suppresses the activity of the enzyme by 36-43%. We observed an analogous pattern with the glyco part (*2*) of the NGP. It is interesting that in all the doses used (5.0, 7.5, 10.0 μg) the carbohydrate part suppressed the activity of the RNA polymerase more strongly than did the protein part. It is not excluded that the carbohydrate part may participate as mediator in protein–nucleic acid interactions. The strongest suppressing action was exhibited by a dose of 7.5 μg both for the peptide and for the carbohydrate moieties.

Thus, the results of the investigations indicate an influence of the NGP on the functional state of the nuclear chromatin. It is important to note that deglycosylated fragments of the GP suppress RNA polymerase activity more strongly than the intact GP (see Fig. 3). It follows from the results obtained that both the GP itself and its fragments suppress the RNA polymerase activity of the nuclei in an *in vitro* system.

EXPERIMENTAL

GP synthesized by the nuclei of rabbit brain cell neurons was used [2].

The amino acid composition of the NGP was determined on a Biotronik IC-700 amino acid analyzer (FRG) in the standard system of 24-, 48-, and 72-hour hydrolysis in 6 N HCl. The monosaccharide composition was determined by thin-layer

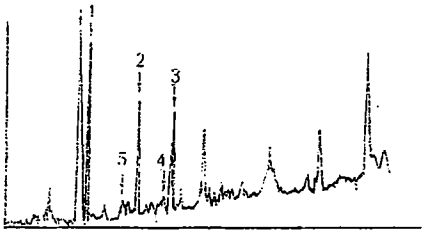

Fig. 2. Spectrum of the monosaccharide composition of the nuclear glycoprotein: *1*) xylose; *2*) mannose; *3*) glucose; *4*) galactose; *5*) galacturonic acid.

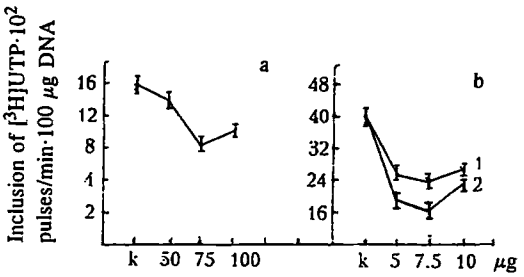

Fig. 3. Action of the glycoprotein and its deglycosylated parts on the RNA polymerase activity of the nuclei of brain neurons: *a*) whole glycoprotein; *b*) deglycosylation fragments: *1*) peptide part; *2*) glyco part.

chromatography. The NG was hydrolyzed with 2 N H_2SO_4 at 100°C for 8-10 h. After neutralization with anion-exchange resin and concentration, the hydrolysate was analyzed by TLC in a fixed layer of KSK silica gel in the systems: 1) butan-1-ol–methanol–water (5:3:1) and 2) butan-1-ol–acetone–water (4:5:1). The chromatograms were revealed with aniline phthalate and the sugars were identified by comparison with standard chromatograms of monosaccharides.

The quantitative levels of neutral monosaccharides in the NPG were determined by V. Markin in the laboratory of carbohydrate chemistry of the Shemyakin Institute of Bioorganic Chemistry, Russian Academy of Sciences, by GLC on a Hewlett-Packard instrument (USA) with a flame-ionization detector. The carrier gas used was helium at an inlet pressure of 1.8 atm. Temperature regime: 100°C for 4 min and then a rise in the temperature to 220°C at the rate of 4°C per minute; capillary column, 0.24 × 40 mm, with the phase SE-30. The sample (200 μg of glycosides) was methylated in 1.5 N HCl in absolute methanol under argon in sealed ampuls at 100°C for 24 h. After this, two equivalents of pyridine + acetic anhydride (200 μl of methanolysis mixture +30 μl of pyridine +40 μl of acetic anhydride) was added and the whole was evaporated.

Trifluoroacetyl derivatives of the methyl glycosides were obtained as described in [8]. To determine the neutral sugar components of the GP, 100 μg of the NGP was hydrolyzed in 0.5 ml of a 0.1 N solution of H_2SO_4 at 80°C for an hour, and, after cooling, 3 ml of a 0.1% solution of anthrone (Calbiochem, USA) in concentrated sulfuric acid was added. The mixture was stirred in the boiling water bath for 15 min, and, after cooling, the absorption at 620 nm was determined (a calibration curve was plotted for glucose). Sialic acid contents were determined by Svennerholm's method [3].

The deglycosylation of the NPG was achieved with the aid of anhydrous HF [9]. The purified GP (1.0 mg) was treated with 0.03 mg [sic] of dry methanol and 0.27 ml of anhydrous 7% HF in pyridine at room temperature for 90 min. The reaction was stopped with 1.0 ml of water and the residual HF was eliminated by dialysis. The deglycosylated material was centrifuged at 1000 rpm for 15 min, after which the glyco part was found in the supernatant and the peptide part of the GP, with no degradation of its structure, in the deposit. After freeze-drying, the glyco and peptide parts were used for further work.

The total RNA-polymerase activity of nuclei was determined as in [5]. The time of incubation at 37°C was 30 min, and the reaction was stopped by the addition of an equal volume of cooled 10% TCAA. The precipitates were transferred to nitrocellulose filters (Synpor, Czech Republic) and, after the filters had been washed with 5% TCAA and 90% ethanol, radioactivity was counted in a Beckman LS-230 scintillation counter. The level of synthesis of RNA was judged from the inclusion of [^{3}H]-UTP in the acid-soluble fraction. The DNA in the samples was determined by Spirin's method [4].

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