COMPOSITION OF THE NEUTRAL SUGARS IN THE PROTEINS SYNTHESIZED *IN VITRO* BY BRAIN AND LIVER NUCLEI

O. Kh. Saitmuratova, D. Akhmedova, and G. Alimkhodzhaeva

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With age, and also in pathological states of the human brain, changes take place in the amount and composition of the glycoproteins it contains [1, 2]. Thus, the amount of glycoprotein isolated from a glial brain tumor was 6-9 times greater than its amount in the brain of healthy subjects [2]. The glycoprotein from cattle brain is a specific antigen for nervous tissue. In the Tay-Sachs disease its amount rises sevenfold [3].

The functional role of the brain glycoproteins has so far been little studied. It is known that some glycoproteins isolated from mouse brain slow down the rate of elongation of polypeptide chains and mitosis in some cell lines [4].

We have previously established that the nuclei of the brain neurons of rabbits *in vitro* synthesize glycoproteins — two water-soluble fractions and two fractions soluble in Tris-glycine buffer. The compositions of the neutral sugars of one of the buffer-soluble fractions of glycoproteins has been given previously [5].

Having investigated the composition of the neutral sugars of the other buffer-soluble fractions of the glycoproteins synthesized by the neurons, we have compared its composition with the buffer-soluble glycoprotein synthesized by liver cell nuclei. Their molecular weights are similar (25,000-30,000).

For the experiment we took the brains and livers of 15-day-old rabbits of one litter immediately after sacrifice. The nuclei of the brain neurons were isolated as described previously [5], and those of the liver by the generally adopted method [7]. The synthesis of protein in the nuclei of the brain and of the liver was carried out under the usual conditions. The extraction of the labeled proteins and glycoproteins, and their fractionation on Sephadex G-50 and hydrolysis were carried out as described previously [5, 6, 8]. In the thin-layer separation of the sugars on silica gel, the butan-1-ol—acetone—water (4:5:1) system proved to be suitable for the brain glycoproteins, and butan-1-ol—methanol—water (5:3:1) for the liver glycoproteins. The results of the investigation show that the Tris-glycine fractions of brain glycoproteins I and II that were studied contained the same monosaccharides — xylose and glucose — the amount of xylose being greater than that of glucose in both fractions. The neutral sugars of the glycoproteins of the liver cell nuclei included xylose, mannose, and glucose residues. Judging from stainability, the amount of glucose exceeded the amount of mannose and glucose. It must be mentioned that xylose and glucose were detected in the nuclear glycoproteins of the brain and the liver neurons, and mannose only in the nuclear glycoproteins of the liver.

Thus, it may be concluded that the glycoproteins synthesized *in vitro* by brain and liver neurons are similar in molecular weight but differ with respect to their neutral sugar compositions.

It is extremely likely that the differences in the composition of the brain and liver nuclear glycoproteins are responsible for their functional specificity.

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Institute of Bioorganic Chemistry, Academy of Sciences of the Uzbek SSR, Tashkent.
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ENZYMATIC-CHEMICAL TRANSFORMATION OF PORCINE INSULIN INTO HUMAN INSULIN USING THE TRYPTIC TRANSAMIDATION REACTION

Yu. P. Shvachkin, E. N. Voluiskaya,
S. P. Krasnoshchekova, and A. M. Nikitina

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We have performed the enzymatic-chemical conversion of porcine insulin into human insulin by a two-stage scheme involving the use of the enzymatic transamidation reaction.

The first stage of the process consists in the trypsin-catalyzed transamidation of the porcine insulin [I, R = de-Ala^{B30}-(porcine insulin)], which takes place when the latter reacts with a L-threonine ester (II, R' = Alk, ArAlk) in an aqueous organic medium (water—ethanol or water—dimethylformamide) at 25°C and pH 6.3. Under these conditions the enzymatic transamidation reaction takes place only at the Lys^{B29} residue and the undesirable side reaction at the Arg^{B22} residue does not take place.

The second stage of the process consists in the chemical demasking of the insulin ester (III) formed in the first stage and has the aim of the exhaustive elimination of the C-protective groupings from the Thr^{B30} residue. The ester derivative (III) was first purified by ion-exchange chromatography on QAE-Sephadex A-25. In the case of the tert-butyl ester of insulin (III, R' = Bu^t), demasking was carried out by treating it with trifluoroacetic acid at 20°C in the presence of anisole as protector. The human insulin formed after acidolysis [IV, R = de-Thr^{B30}-(human insulin)] was isolated from the reaction mixture with the aid of gel filtration of Sephadex G-25 fine. The course and degree of the transformation was monitored by TLC on silica gel, electrophoresis on cellulose, and disk electrophoresis in polyacrylamide gel.

After lyophilization of the eluate, we obtained human insulin (IV) in analytically pure form.

The electrophoretic mobility of compound (IV) was 1.35 (electrophoresis on Whatman No. 1 paper, pH 1.9, 450 V, 7 mA, staining by the Pauli reagent, reference standard the bis-S-sulfonate of the B-chain of human insulin). Amino acid analysis: Asp 3.00 [3], Thr 2.85 [3], Ser 3.20 [3], Glu 7.10 [7], Pro 1.20 [1], Gly 3.80 [4], Ala 0.98 [1], Cys 5.15 [6], Val 4.10 [4], Ile 1.85 [2], Leu 6.00 [6], Tyr 3.35 [4], Phe 2.85 [3], His 1.92 [2], Lys 0.95 [1], Arg 0.98 [1]. The results of the determination of the C-terminal amino acids: Thr 0.98 [1], Asn 0.83 [1].

The proposed method for the enzymatic-chemical transformation of porcine insulin can be extended to the insulins of other biological species and permits the production not only of human insulin but also of various structural analogs of this hormone containing other amino acids in place of the Thr^{B30} residue and also fragments of their various derivatives including peptides.

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