Gas chromatography analysis of sugars in glycoproteins of blood serum

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A gas chromatography method of simultaneous determination of the total content of monosaccharides and amino sugars in glycoproteins of blood serum is described. The chromatographic analysis of the trimethylsilyl ethers of sugars was performed after a combined enzymatic and acid hydrolysis of glycoproteins. The values of retention times, response factors, and concentrations of sugars in human, rabbit, and horse blood serum are presented.

Описывается метод газовой хроматографии для одновременного определения общего содержания моносахаридов и аминосахаров в гликопротеинах сыворотки крови. Хроматографический анализ триметилсилил эфиров сахаров осуществился после комбинированного энзиматического гидролиза гликопротеинов в кислой среде. Приводятся значения времен удерживания, поправочных коэффициентов и концентрации сахаров в сыворотке крови человека, кролика и лошади.

Glycoproteins occur widely in nature. They consist of a protein component and sugar covalently bonded to it. Glycoproteins constitute the major part of plasma proteins, since approximately sixty out of the total number have been well characterized and isolated in the pure form [1]. In the study of biological roles of glycoproteins it is important to pay attention to sugars as well as proteins. The predominant monosaccharides of these macromolecules are: *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, D-galactose, D-mannose, L-fucose, *N*-acetyl-D-neuraminic acid, and rarely D-xylose. The carbohydrate contents of human plasma proteins with detailed data are known for a great number of glycoproteins isolated from plasma [2].

The development of gas—liquid chromatography has recently been demonstrated in the quantitative analysis of carbohydrate content of the glycoproteins. The sugars in glycoproteins are most frequently determined in the form of acetates, alditol acetates, trifluoroacetates, and trimethylsilyl ethers [3—7]. The monosaccharide units as well as amino sugars are usually obtained by 6—12 hours'

hydrolysis of glycoproteins at boiling temperature using 1 N hydrochloric or sulfuric acid. A mild and shortened hydrolysis following a pepsin digestion was adopted for human immunoglobulins IgG and IgM [8]. In this communication we describe a method for the determination of monosaccharides and amino sugars in glycoproteins for the three above-mentioned blood sera.

Experimental

The sugars used in this investigation as references were A.R. grade from Sigma Chemical Co., USA.

Hexamethyldisilazane, trimethylchlorosilane, and pepsin were anal. grade (Lachema, Brno).

Gas chromatography of the TMS ethers of sugars was carried out on an instrument Fractovap 2200 (Carlo Erba, Milan). The glass column of the length of 220 cm was packed with Chromosorb G AW DMCS of the grain size of 100—120 mesh coated with 5% NGS (neopentylglycol succinate) and 3% Apiezone N. The flow of nitrogen used as a carrier gas was 80 ml/min at the temperature programme 5°C/min within the range 140—230°C. The detection was performed by means of flame ionization detector and the values of concentrations of the respective sugars were obtained by using a Minigrator (Spectra Physics, USA) and D-arabinose as an internal standard.

The samples of sugars for chromatography were obtained from a blood serum (0.3 ml) which was precipitated with 20% trichloroacetic acid. After washing with distilled water the precipitate was suspended in distilled water (2 ml). Then 0.5 ml of pepsin solution (30 mg of pepsin per 100 ml of distilled water) and a few drops of concentrated HCl were added into the suspension. The pepsin hydrolysis took place in a closed flask at 37°C for 12 h. After the enzymatic digestion 2.5 N-HCl (3 ml) was added to the solution. Then the acid hydrolysis was carried out for 10 h at boiling temperature in sealed ampules. The hydrolysate was neutralized by calcium hydrogen carbonate and then 0.2 ml of the solution of arabinose (42.5 mg) in pyridine (25 ml) was added. After evaporation the residue was dissolved in anhydrous pyridine (2 ml), and hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.2 ml) were added to afford the trimethylsilyl ethers of sugars. The reaction mixture was bubbled through with nitrogen to remove the solvent and the residue was dissolved in pyridine and subjected to chromatographic analysis.

Results and discussion

The relative retention times and response factors of TMS derivatives of L-arabinose, L-fucose, D-mannose, D-galactose, D-glucose, D-galactosamine, and D-glucosamine are presented in Table 1. The persilylation of these sugars proceeds quantitatively in anhydrous pyridine, this being confirmed by the fact that only separate peaks were obtained in gas chromatography (Fig. 1).

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Table 1

Retention times, response factors, and concentrations of monosaccharides and amino sugars obtained in analyses of human, rabbit, and horse blood serum

Carbohydrate	Response factor	Retention time		Concentration mg/100 ml		
		min	related to arabinose	Man (n=20)	Rabbit (n=12)	Horse (n=12)
L-Arabinose	1.00	13.1	1.00	—	—	—
L-Fucose	1.04	15.5	1.18	25.9±0.010	28.3±0.012	11.5±0.004
D-Mannose	0.88	17.0	1.29	33.4±0.063	29.0±0.258	30.1±0.054
D-Galactose	0.65	18.7	1.42	36.2±0.238	17.1±0.085	33.9±0.223
D-Glucose	1.13	20.0	1.53	20.4±0.006	13.1±0.004	4.5±0.001
D-Glucosamine	0.77	30.2	2.30	41.2±0.074	34.2±0.074	26.7±0.040
D-Galactosamine	0.59	32.5	2.48	6.8±0.023	3.4±0.011	9.6±0.032

Standard deviations were calculated according to Student's t-distribution.
n = number of analyses.

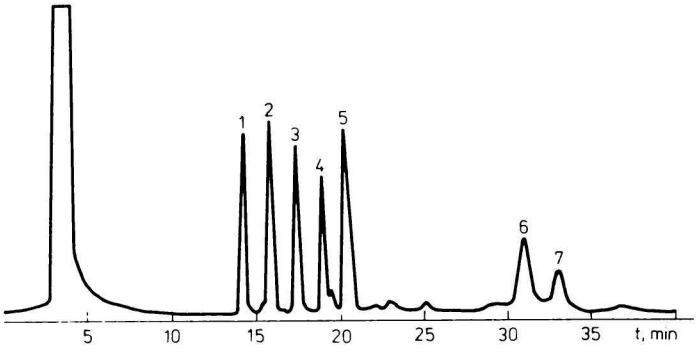

Fig. 1. Gas chromatography of the TMS ethers of sugars on a column with NGS and Apiezone N at 140—230°C and nitrogen flow 80 ml/min.
Peaks: *1.* L-arabinose; *2.* L-fucose; *3.* D-mannose; *4.* D-galactose; *5.* D-glucose; *6.* D-glucosamine; *7.* D-galactosamine.

In the combined enzymatic and acid hydrolysis a mixture of monosaccharides and amino sugars is formed from glycoproteins. The liberated amounts of individual sugars are proportional to the concentration of glycoproteins in blood serum because the composition of the sugar component of glycoprotein is constant and given by the structure of this substance. Therefore, the values of the sugar concentrations obtained by the hydrolysis of serum must depend on the qualitative and quantitative composition of glycoproteins in serum. The chromatography of TMS ethers of sugars obtained after a combined enzymatic and acid hydrolysis of human blood serum is shown in Fig. 2.

An excessive pepsin digestion or acid hydrolysis results in the existence of disturbing chromatographic peaks which are likely due to the liberated amino

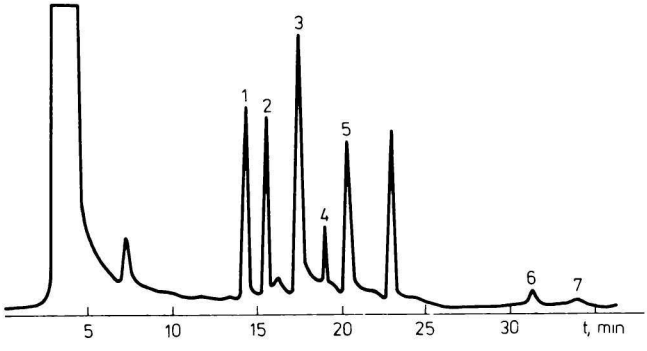

Fig. 2. Gas chromatography of the TMS ethers of monosaccharides and amino sugars obtained by the combined enzymatic and acid hydrolysis of human blood serum.
Denotation of peaks as in Fig. 1.

acids. The experimental conditions of hydrolysis and adequate chromatographic separation enable us to evaluate a chromatographic record with sufficient accuracy.

The proportions of L-fucose, D-mannose, D-galactose, D-glucose, D-galactosamine, and D-glucosamine in the human, rabbit and horse blood sera are relatively different, which is due to the total content of glycoproteins in the sera of individual species as well as their qualitative composition and structure (Table 1). As to D-glucose, some authors [9] explain the presence of this substance in pure glycoproteins by assuming the contamination in the course of preparation procedures, which is out of the question in the case of the determination according to our method. Anyway, it would be convenient to relate the presence of D-glucose with its role of major precursor in biosynthesis of individual glycoproteins.

The described chromatographic method is suitable for the determination of monosaccharides and amino sugars in human, rabbit, and horse serum glycoproteins containing approximately 5% of carbohydrates. On the basis of high sensitivity and reproducibility it is possible to recommend this method in the biochemical study of glycoproteins.

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