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Note

Separation of amino sugars and amino acids in glycoproteins and biological fluids on resin-coated chromatoplates

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In this paper, a method is described for the separation of hexosamines and aromatic and basic amino acids from hydrolyzates of glycoproteins and glycopeptides and in biological fluids. The separation is achieved by using resin-coated chromatoplates for thin-layer chromatography and 1–2 nmoles of hexosamines in the samples investigated can be determined. The main difference between this method and those published earlier lies in the different ionic strength and pH of the buffer solution used in the elution procedure.

Large amounts of amino sugars can be separated on a cation-exchange resin column or on an amino acid analyzer[3–6]. Recently, the separation of seven hexosamines has been described[7]. Some methods are available for separating small amounts of hexosamines and amino acids by using partition thin-layer or paper chromatography[8–10], but the separations are not satisfactory. The sensitivity of specific hexosamine reagents[11–13] is less than that of ninhydrin, but it cannot be used in the presence of amino acids because of the unsatisfactory separation obtained.

The amino acids in biological fluids and in acid hydrolyzates of peptides and proteins can be separated on resin-coated chromatoplates[1,2,14]. Recently, basic water-soluble antibiotics have been separated by using a sodium acetate buffer containing NaCl and *tert.*-butanol[15]. However, the amino sugars derived from glycoproteins or glycopeptides and biological fluids cannot be separated from amino acids in these buffers.

EXPERIMENTAL AND RESULTS

Ionex 25-SA-Na^+ resin-coated chromatoplates (20 × 20 cm; Macherey, Nagel and Co., Düren, G.F.R.) were used, and in some experiments Fixion 50-X8-Na^+ resin-coated chromatoplates (Chinoin, Budapest). The chromatoplates were equilibrated before use[1].

All solutions were prepared by using demineralized water. All reagents were of analytical grade.

The hydrolysis of glycoproteins and glycopeptides was carried out in 4 *N* HCl at 105° for 14–24 h. The hydrolyzates were filtered and 10–20-μl volumes were applied directly to the chromatoplates by using a calibrated glass capillary. The samples from biological fluids were prepared according to Dévényi[1] and Dévényi *et al.*[14].

The R_F values of the hexosamines and the basic and aromatic amino acids at several pH values and Na^+ concentrations are shown in Tables I and II. It is evident that the most favourable separation is achieved by using a buffer of pH 5.28 and 0.26 *M* sodium citrate concentration.

TABLE I

R_F VALUES OF AMINO SUGARS AND AMINO ACIDS AT DIFFERENT pH VALUES

	R_F *in 0.3 M* Na^+			
	pH			
	*3.0**	*4.0***	*5.0*	*6.0*
Leucine	1.00	1.00	1.00	1.00
Phenylalanine	0.57	0.60	0.66	0.64
Glucosamine·HCl	>1.00	0.97	0.58	0.58
Galactosamine·HCl	>1.00	0.85	0.49	0.51
Lysine·HCl	0.14	0.23	0.32	0.36

* The spots of hexosamines cannot be separated from those of neutral amino acids.
** The spots of hexosamines cannot be separated from that of leucine.

TABLE II

R_F VALUES OF AMINO SUGARS AND AMINO ACIDS AT DIFFERENT Na^+ CONCENTRATIONS

	R_F *at pH 5.28*					
	Na^+ *concentration (M)*					
	0.2	*0.25*	*0.28*	*0.30*	*0.35*	*0.40*
Leucine	1.00	1.00	1.00	1.00	1.00	1.00
Phenylalanine	0.67	0.67	0.66	0.63	0.57	0.51
Glucosamine·HCl	0.50	0.56	0.57	0.55	0.53	0.50
Galactosamine·HCl	0.47	0.49	0.50	0.50	0.49	0.46
Lysine·HCl	0.28	0.31	0.32	0.33	0.33	0.35

The eluting buffer contained 18.2 g of citric acid monohydrate, 10.4 g of sodium hydroxide, 4.7 ml of concentrated HCl (sp. gr. 1.19) and demineralized water to 1000 ml.

The blood samples, if they were deproteinized with trichloroacetic acid, were pre-chromatographed in 0.01 *N* HCl in order to remove the trichloroacetic acid[1]. Other samples were developed in the sodium citrate buffer by the ascending technique, without pre-chromatography, until the level of the buffer reached the top of the plate. After chromatography the plates were dried and the spots were rendered visible with a collidine–ninhydrin spray reagent[2]. A typical chromatogram of the hydrolyzates of glycoproteins and glycopeptides is shown in Fig. 1.

The hexosamine content of the samples was measured by the methods of Boas[16] and Dische and Borenfreund[17]. The results of hexosamine analysis are summarized in Table III. It is evident that less than 0.5 μg of amino sugar (about 1–2 nmole) can be detected on the plates.

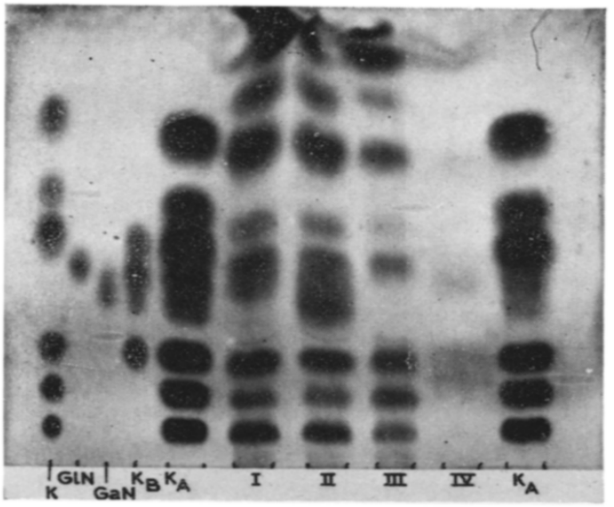

Fig. 1. Detection of amino sugars and aromatic and basic amino acids on a cation-exchange resin coated chromatoplate. Eluting buffer: sodium citrate, pH 5.28, 0.26 M Na^+. Samples are as follows. K: amino acid control mixture containing, in order of increasing R_F values, Arg, His, Lys, Phe, Tyr and Leu. K_A: control mixture, containing, in order of increasing R_F values, the amino acids mentioned above and amino sugars, viz., Arg, His, Lys, GaN (galactosamine·HCl), GlN (glucosamine·HCl), Phe, Tyr and Leu. K_B: mixture of Lys, GaN, GlN and Phe. I: egg protein hydrolyzate, containing GlN. II: egg protein hydrolyzate+GaN. III: hydrolyzate of a protein, not containing amino sugars. IV: hydrolyzate of a glycopeptide, containing GlN, prepared from egg protein.

TABLE III

RESULTS OF THE HEXOSAMINE ANALYSIS

Sample	*Hexosamine content*		
	μg per ml of hydrolyzate		*ng per 10 μl of hydrolyzate*
	Boas method	*Dische–Borenfreund method*	*Average*
Egg protein (I in Fig. 1)	23.7	21.0	223
Protein (III in Fig. 1)	Not measurable	Not measurable	—
Glycopeptide	16.2	19.9	180.5

CONCLUSIONS

A new method has been developed for determining the amino sugars and aromatic and basic amino acids in biological fluids and glycoproteins. Using this method, the sensitive ninhydrin reagent can be applied in order to render visible the spots of the amino sugars and amino acids. The spots have different colours if the ninhydrin spray contains collidine. The quantitative analysis of the samples can be carried out with an automatic amino acid analyzer[5,6], or by specific colorimetric methods[16–19].

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