STRUCTURAL AND IMMUNOLOGICAL STUDIES ON A SIALOGLYCOPEPTIDE ISOLATED FROM HUMAN URINE

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SUMMARY : A sialoglycopeptide is isolated from human pregnancy urine after gel filtration, ion-exchange chromatography, paper chromatography, and high voltage paper electrophoresis. It contains serine, N-acetyl-galactosamine, galactose, sialic acid (1-1-1-2). Structural studies show that the carbohydrate moiety is likely O-glycosidically linked to serine, and related to MN blood group structures. However this glycopeptide does not exhibit any cross antigenic reactivity by the hemagglutination assay using anti-N, anti-M rabbit immune sera or anti-N lectin from Vicia Graminea.

INTRODUCTION

Human urine contains a wide range of low-molecular-weight carbohydrate-rich compounds (1). They are glycopeptides and oligosaccharides which can be shared into fuco- and sialoconjugates by DEAE cellulose chromatography. Up to now few sialoconjugates have been obtained from human urine. In the laboratory, three glycopeptides (2-4) have been isolated and some of their structural features described. With regard to sialosaccharides, Huttunen (5) has isolated and completely characterized four sialooligosaccharides, one of them having the same structure than the carbohydrate moiety of MN blood group substances.

This paper reports the isolation and partial structure of a new urinary sialoglycopeptide. According to the similarities between the carbohydrate moieties of this sialoglycopeptide and of MN blood group substances, it is looked after any possible blood group activity.

MATERIALS AND METHODS

1. Preparation of the sialoglycopeptide

The purification of the sialoglycopeptide was carried out as shown

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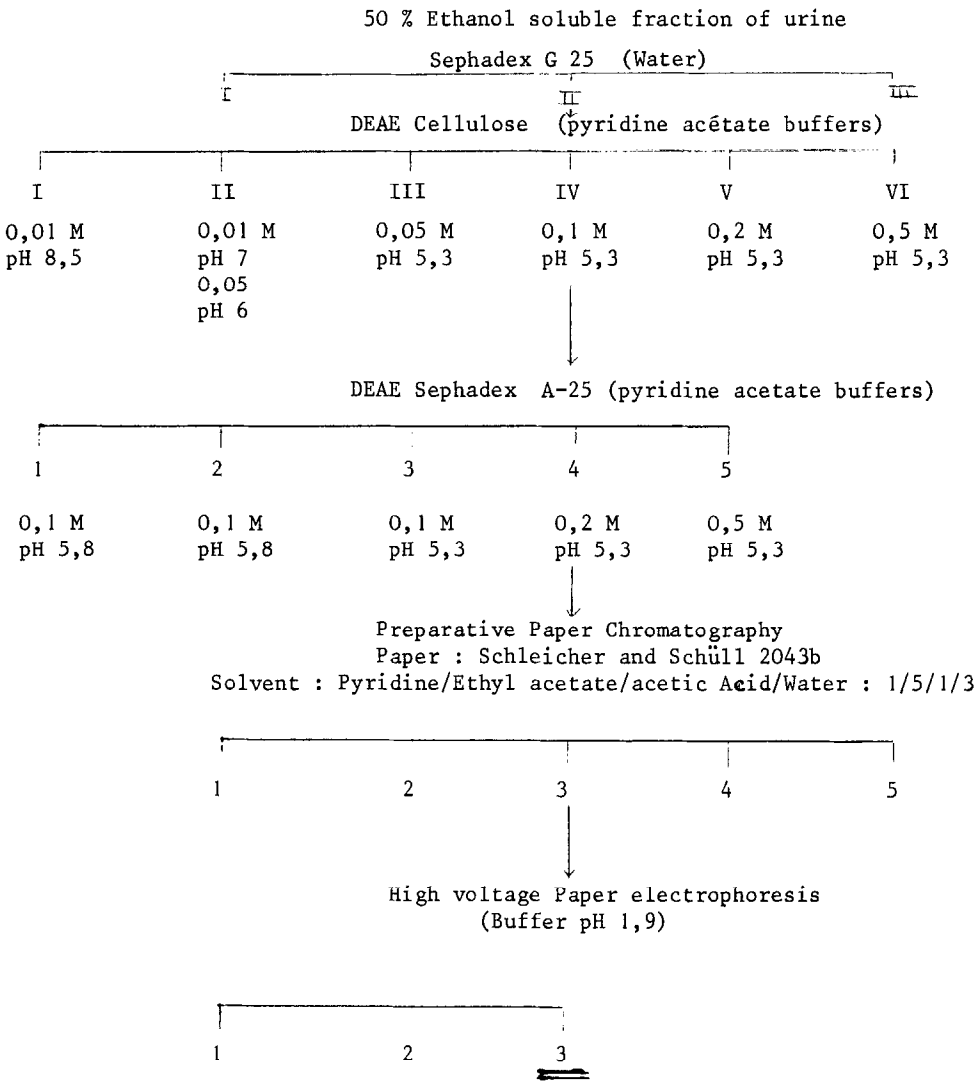
in fig. 1. It is homogeneous in gel filtration and in high voltage paper electrophoresis at pH 1,9 after staining with ninhydrin and Schiff's reagent after a periodate oxydation.

Fig. 1 Purification of the sialoglycopeptide



2. Analytical techniques

Aminoacids were determined with the Beckman aminoacid analyser before and after a 6 N HCl, 7 h hydrolysis, under vacuo.
Hexoses were determined by the orcinol sulfuric acid method (6) and by gas-liquid chromatography (GLC) (7).

Hexosamines were determined with the Beckman aminoacid analyser Unichrom (8) and by a colorimetric assay (9) after a 3 N HCl, 4 h hydrolysis, and by GLC (7). Sialic acid was quantitated by GLC (7) and according to Warren (10) after a mild acid hydrolysis 0,1 N H_2SO_4, 60 minutes, 80°C. The quantitative desialization of the native glycopeptide was carried out by a 0,1 N H_2SO_4, 80°, 1 h hydrolysis. After a ten fold dilution the solution was filtered through a Dowex 1 x 4 (Acetate form) column in water. The material eluted by water was freeze dried and submitted to carbohydrate analysis by GLC. No loss of galactose was observed and all the sialic acid was splitted. In 24 hours, Neuraminidase (EC 3. 2. 1. 18) from Clostridium perfringens grade V (Sigma) liberated only 57 % of the sialic acid.

β Galactosidase (EC 3. 2. 1. 23) of Jack Bean meal was prepared in the laboratory according to Li (11). β Galactosidase (EC 3. 2. 1. 23) of beef liver was purchased from Sigma. Native and desialized glycopeptide (1 μ M) was incubated with 18 units of the Jack bean β galactosidase (citrate buffer pH 4,6, 0,05 M) and 18 units of the beef liver β galactosidase (phosphate saline buffer pH 7,2 0,2 M (NaCl 0,1 M) for 60 h at 37°C. The released galactose was determined enzymatically (12).

For the study of alcali-labile bonds, the desialoglycopeptide (2 μ M) was incubated in 0,1 M NaOH and 0,4 M Na BH_4 for 40 hours at 37°C. Excess borohydride was destroyed by the addition of acetic acid, and boric acid was removed as methyl borate. Hexosamines, hexosaminitols and amino acids were determined with the amino acid analyser before and after a 3 N HCl 4 hours hydrolysis.

The hemagglutination inhibition assays were performed according to Font et al (13). The M and N erythrocytes were obtained from the Centre National de Transfusion Sanguine (Paris). The anti-N (Lot N 118) and anti-M (Lot M 114) rabbit immune sera were obtained from Ortho Diagnostics (Paris). The anti-N Vicia Graminea lectin was prepared in the laboratory by M.J. Pringent.(14). The Cow K caseino-glycopeptide (1() was used as a reference. Each assay was run twice with both native and desialized glycopeptide and K caseinoglycopeptide.

RESULTS AND DISCUSSION

The composition of the sialoglycopeptide is given in table 1. The acid hydrolysis liberates serine and galactosamine so that a O-glycosidic linkage is likely. However, after a alkali-borohydride treatment, followed by acid hydrolysis, no loss of serine and of galactosamine was observed as well as no alanine or galactosaminitol had appeared. When the alkali-borohydride treatment was not followed by an acid hydrolysis, the glycopeptide was excluded from the amino acid analyser resin after the same time (24,8 minutes ; 53 minutes for the serine) than the non alkali-treated glycopeptide. This failure for the glycopeptide to undergo β elimination is explained by the fact that both amino and carboxyl groups of the serine are unsubstituted so that intermediate anions are stabilized (16-17), preventing carbohydrate moiety from elimination.

The results of the incubations with various β galactosidases is given in table 2. Desialyzation is needful to allow β galactosidases hydrolysis. Further it can be assumed that galactose is β 1→3 linked to N-acetyl galactosamine as the β galactosidase from Jack Bean is known to free galactose from its β 1→3 linkages very slowly (18).

Table 1 Chemical Composition of the Glycopeptide

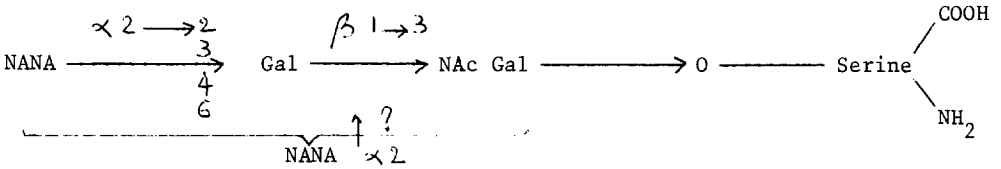
		Molar ratio to serine
Galactose	GLC*	0,92
	Colorimetric assay	1,25
NH_2Galactosamine	GLC* (NAc Gal)	0,75
	Amino acid analyser*	0,82
	Colorimetric assay	0,8
NANA	GLC	2,1
	Colorimetric assay	1,83
Serine	Amino acid analyser*	1
Other components Aminoacids or Carbohydrates		$< 0,10$

* determined on both sialo and desialo glycopeptide

Table 2 : Release of galactose by galactosidases (18 units) expressed as mole by mole of glycopeptide

	Jack Bean (pH 4,6, 0,05 M) citrate buffer	Beef liver (pH 6, 0,02 M) phosphate saline buffer NaCl 0,1 M
Sialoglycopeptide	0	0
Desialized glycopeptide	0,09	0,33

A likely partial structure of this glycopeptide is then proposed



This carbohydrate structure is very closed to those reported for M and N blood group antigens obtained from M and N erythrocytes (19-20). It was then of interest to look for any cross antigenic reactivity between this sialoglycopeptide and M and N blood group substances. The inhibition tests show that in the same conditions (15) the urinary sialo and desialo glycopeptide does not exhibit any N or M blood group activity whereas the Cow K caseinoglycopeptide presents a cross antigenic reactivity with the N blood group substances. It must be noticed that it has not been looked for immunological activities of the urinary saccharide (5) and for the saccharides obtained by alkali-borohydride treatment of erythrocyte glycopeptides (19-20). These results agree with the findings of Pusztaï and Morgan (21) who showed that proteolysis of blood group substances caused a marked loss of their blood group activities. Further, our results also agree with the hypothesis of Lisowska, that the presence of a lysine residue in the peptidic chain should be involved in MN blood group activity (22). Though this new urinary sialoglycopeptide does not exhibit any cross-antigenic reactivity with MN blood group substances, it can be assumed that it likely originates from erythrocyte glycoproteins.

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