OLIGOSACCHARIDES FROM M-ACTIVE SIALOGLYCOPEPTIDES OF HUMAN ERYTHROCYTES

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Summary

A sialoglycopeptide released from human type M erythrocytes by incubation with trypsin was treated with 0.1 M NaOH containing 0.4 M $NaBH_4$. Nine low molecular weight sugars and oligosaccharides were released, including four which had N-acetylgalactosaminitol at the "non-reducing" terminus. All of these appear to be derived or derivable from a tetrasaccharide containing two sialic acids, one galactose and one galactosamine with the latter linked O-glycosidically to serine or threonine.

Sialoglycopeptides with M or N bood group activity are liberated from the surface of human erythrocytes on incubation with trypsin (Mäkela et al, 1960; Cook et al, 1960; Eylar et al, 1962). A previous report (Winzler et al, 1967) has established that such glycopeptides have a portion of their carbohydrate linked to the peptide chain by alkali-labile, O-glycosidic bonds. In the present paper the nature of the oligosaccharides released by treating sialoglycopeptides with alkaline borohydride will be considered.

Materials and Methods.

Cells were typed for their MM, NN, MN serological specificity using commercially available antisera. Sialoglycopeptides were obtained from the typed cells by treatment with trypsin as described previously (Winzler et al, 1967). Hexose was determined by the phenol-sulphuric acid method (Dubois et al, 1956) or by gas-liquid chromatography of the alditol acetates (Lehnhardt and Winzler, 1968). Sialic acid was determined by the direct Ehrlich reaction (Werner and Odin, 1952) or by the thiobarbituric acid method (Warren, 1959). Hexosamines and hexosaminitols were determined with the amino acid analyzer (Weber and Winzler, 1969). Sialic acid was liberated from its glycosides with neuraminidase (Cassidy et al, 1965) or by "mild acid hydrolysis" (0.1N HCl, 80°, 1 hour). Incubations with β-galactosidase from Phaseolus vulgaris were as described by Agrawal and Bahl (1968). Descending paper chromatography was performed with Whatman No. 3 paper and two solvent systems (A) 1-butanol-pyridine-water (6:4:3 v/v (B) 1-butanol-acetic acid-water (4:1:5 v/v). Sugars and amino sugars on paper chromatograms were detected with the periodate-benzidine spray reagent (Gordon et al, 1956). For the cleavage of alkali-labile bonds, sialoglycopeptides (1 % solution) were incubated in 0.1 M NaOH - 0.4 M $NaBH_4$ for 26 hours at room temperature in

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Abbreviations: Nacetyl galactosaminitol, 2-acetamido-2-deoxy-D-galactitol. NANA., Nacetyl neuraminic acid.

sealed tubes, in the dark under nitrogen. Excess borohydride was destroyed by the addition of acetic acid, and the mixture was fractionated on a Sephadex G-25 column. The retarded fractions of the G-25 eluate, corresponding to low molecular weight oligosaccharides, were pooled and passed through a column of Dowex 50 x 8 (H^+ 200-400 mesh). The eluate was lyophilized under reduced pressure, and boric acid was removed as methyl borate. The oligosaccharides were fractionated further on a Sephadex G-15 column and by repeated preparative paper chromatography in the above solvents.

Results and Discussion.

When M-active sialoglycopeptides were incubated in alkaline borohydride solution under the conditions described previously (0.1 M NaOH - 0.2 M $NaBH_4$, room temperature, 48 hours) (Winzler et al, 1967), several oligosaccharides containing unsaturated sugar residues were released, as shown by:

(a) their ability to decolorize alkaline potassium permanganate or 0.2% bromine water.

(b) production of glycerol after ozonolysis, reduction and acid hydrolysis.

(c) presence of unidentified alditol acetate peaks in the gas chromatograph after reduction with hydrogen in the presence of platinum followed by acid hydrolysis. Therefore, conditions were modified to obtain oligosaccharides with the minimum of alkaline degradation (see methods). In the revised procedure 25 - 30% of the hexose was released as low molecular weight

TABLE I.

Oligosaccharide Component		Molar Ratios	Rlactose; Solvent A.
Fraction I.	Sialic Acid	2.2	.230
	Galactose	1.1	
	N-Acetyl Galacto-saminitol	1.0	
Fraction II.	Sialic Acid	1.0	.540
	Galactose	1.08	
	N-Acetyl Galacto-saminitol	1.0	
Fraction III.	Sialic Acid	1.0	.620
	Galactitol	1.08	
Fraction IV.	Sialic Acid		.820
Fraction V.	Galactose	1.2	1.40
	N-Acetyl Galacto-saminitol	1.0	
Fraction VI.	Galactitol		1.67
Fraction VII.	N-Acetyl Galacto-saminitol		2.0
Fraction VIII.	3-Deoxy Glucitol		2.05
Fraction IX.			2.55

material. Unsaturated sugar residues were absent according to the above criteria. Analyses of purified fractions are given in Table I.

Fraction I contained NANA, galactose and N-acetyl galactosaminitol in molar ratios of 2:1:1. Treatment of Fraction I with neuraminidase, or by mild acid hydrolysis,

resulted in the release of sialic acid and yielded material with chromatographic mobility (solvents A and B) and a molar composition identical to Fraction V.

Fraction II (NANA, galactose, N-acetyl galactosaminitol, 1:1:1), when treated with neuraminidase, or after mild acid hydrolysis, also gave rise to Fraction V.

Fraction III contained equimolar NANA and galactitol, and neuraminidase treatment released sialic acid and galactitol which were identified by their chromatographic mobility in solvents A and B.

Fraction IV. This was identified as free sialic acid by its chromatographic mobility and color reaction with the thiobarbituric acid reagent (Warren, 1960).

Fraction V contained equimolar galactose and N-acetyl galactosaminitol and was the only fraction to show activity with galactose oxidase (Avigad et al, 1962) (10-12% of the theoretical value) indicating that carbon atom 6 of galactose was not substituted. Also, it was the only fraction to be cleaved by β-galactosidase, indicating a β-linkage of galactose to N-acetyl galactosaminitol.

Fraction VI behaved as free galactitol on chromatography in solvents A and B and on gas liquid chromatography of the hexaacetate.

Fraction VII was identified as N-acetylgalactosaminitol by its chromatographic mobility and behavior in the amino acid analyzer after acid hydrolysis (2N HCl/100°C/4 hours).

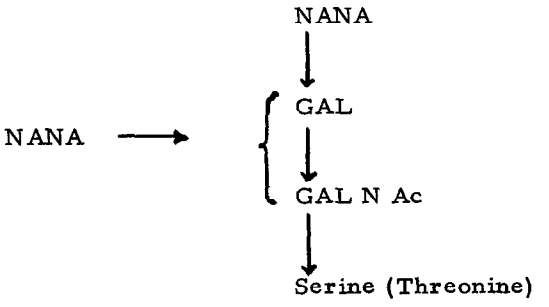
Fraction VIII had the chromatographic mobility

of authentic hexane-D-ribo 1, 2, 4, 5, 6-pentol (3-deoxy-D-glucitol; Rlac = 2.05), and an identical retention time on gas liquid chromatography of the acetate. The product of periodate oxidation was identified as malonaldehyde by the colorimetric procedure of Saslaw and Waravdekar (1959). Lloyd et al (1968) have reported the occurrence of a 3-deoxy-D-glucitol residue in an oligosaccharide released by alkaline borohydride treatment of blood group Le^a substance. It is possible that Fraction VIII is a product from the galactose moiety of one of the above oligosaccharide fractions, e.g. Fraction I or II.

Fraction IX is thought to be a reduced form of the Kuhn chromogen (Kuhn and Kruger, 1956). It gave a positive direct Ehrlich Reaction. The chromatographic mobility in solvent A is identical with that reported by Lloyd et al (1969) for the reduced chromogen obtained by alkaline treatment of disaccharides with the (1⟶3) 2-acetamido-2-deoxy-D-hexose linkage. Also Bray et al (1967) have observed a reduced chromogen product in the amino acid analyzer after treating keratosulphate with $NaOH-NaBH_4$. After acid hydrolysis of Fraction IX (1 N HCl/100°C/4 hours) the product was eluted in the amino acid analyzer at the position reported by Bray et al (1967).

It is probable that Fractions I and II, a tetrasaccharide and a trisaccharide, are the native alkali-labile oligosaccharide residues of the sialoglycopeptides, and that the other fractions are alkaline degradation products thereof.

A likely partial structure for the tetrasaccharide residue is shown in Fig. I.



It is of interest that an oligosaccharide having a molar composition identical with that of Fraction I, but with N-acetyl galactosamine as a reducing end group, has been found in human urine. (Huttunen, 1966).

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