MANNOSE-CONTAINING OLIGOSACCHARIDES ISOLATED FROM α_1-ACID GLYCOPROTEIN OF HUMAN PLASMA

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Mannose has been shown to be present in the inner part of the polysaccharide chains of the α_1-acid glycoprotein (orosomucoid) (Eylar and Jeanloz, 1962; Hughes and Jeanloz, 1964 & 1966; Li, 1966; Sato et al., 1967; Wagh et al., 1966; Willard, 1962). Moreover, the results of the previous investigation (Sato et al., 1967) indicated the presence of C_3-substituted mannose molecules.

In order to verify the above indication, the hydrazinolysate of the α_1-acid glycoprotein of human plasma (Yosizawa et al., 1966) was oxidized with periodate and subsequently reduced with sodium borohydride. After N-acetylation of an acid hydrolysate of the product, mannose-containing oligosaccharides were isolated and characterized.

Isolation of oligosaccharides----A portion (1.5 g) of the hydrazinolysate of the α_1-acid glycoprotein (Yosizawa et al., 1966) was dissolved in 300 ml of chilled 0.1 M aqueous sodium metaperiodate, and kept in the dark for 45 h at 4°. After oxidation, the reaction mixture was treated with 70 ml of 50% aqueous ethylene glycol, and then concentrated and passed through a column (3.1 x 60 cm) of Sephadex G-25, which was subsequently washed with water. The Molisch-positive effluent was treated with sodium borohydride (1.5 g) for 15 h at 4°, followed by

neutralization with acetic acid. The desalted effluent (75 ml) through the Sephadex G-25 column was heated for 1 h at 80° with the same volume of 0.1 N sulfuric acid. The hydrolysate was neutralized with 0.1 N sodium hydroxide, and treated with Sephadex G-25 as above. The Molisch-positive fraction was passed through a column (2 x 15 cm) of Dowex 1 x 2 (acetate), and the effluent was concentrated and lyophilized. The product (430 mg) thus obtained was hydrolyzed with 50 ml of 1 N sulfuric acid for 4 h in a boiling water bath. Subsequently, the hydrolysate was neutralized with 0.3 N barium hydroxide. The substance in the supernatant of the solution was N-acetylated according to the procedure of Carlson et al.(1964). The resulting product, as can be seen in Fig. 1, contained large amounts of N-acetylglucosamine and mannose, small amounts of galactose, glycerol and several oligosaccharides. These oligosaccharides were designated as Oligs. I, II, and III, as described in Fig. 1. Preparative paper chromatography of these compounds, under the same condition as described in Fig. 1, yielded 6.1 mg, 2.0 mg and 0.5 mg of Oligs. I, II, and III, respectively. Being chromatographed with another solvent, ethylacetate-acetic acid-water (2:1:2, v/v, upper layer, kept for about 1 year), Olig. I was separated into two components, Olig. I-a and Olig. I-b, which had $R_{maltose}$ (rate of migration compared to that of maltose) 1.1 and 0.9, respectively. These two components were purified by preparative paper chromatography with this solvent. The aqueous eluant of each compound from the chromatograms was finally treated with a small amount of charcoal (Dalco G-60), and then lyophilized. Olig. I-a, 1.8 mg and Olig. I-b, 4.1 mg, were obtained.

Characterization of oligosaccharides----Knowing from Table I, mannose residue in Olig. I-a was reduced almost quantitatively,

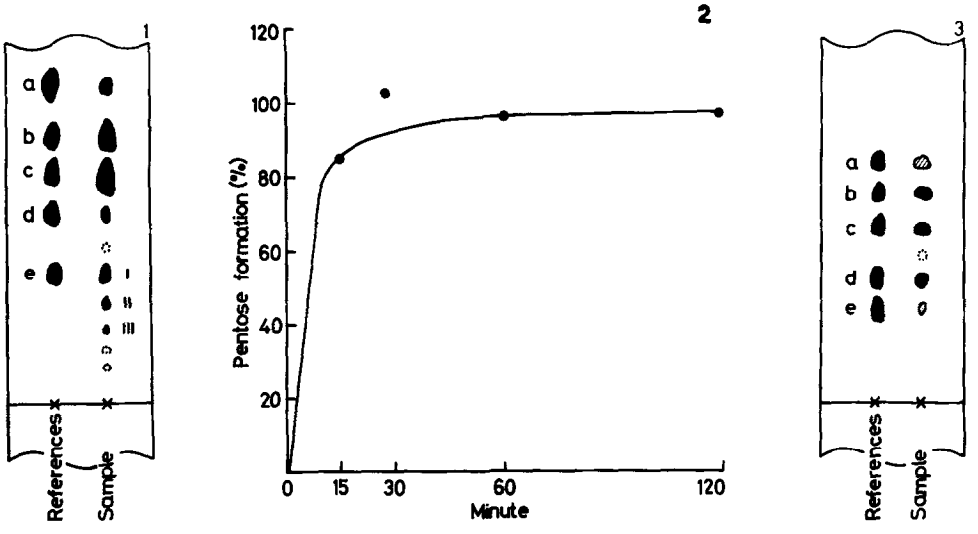

Fig. 1. Paper chromatogram of the N-acetylated, acid hydrolysate of the unoxidized carbohydrate moiety of the α_1-acid glycoprotein of human plasma.

Paper chromatography was carried out on Toyo filter paper No.3 by the ascending technique, using n-butanol-pyridine-water (11:9:6, v/v) solvent, for 30 h at room temperature. Staining reagent, alkaline-silver.

References: a, glycerol; b, N-acetylglucosamine; c, mannose; d, galactose; e, maltose.

Fig. 2. Formation of pentose from Olig. I-b during oxidation with lead tetraacetate.

Oxidation was carried out according to the procedure of Charlson and Perlin (1956): An aqueous solution (0.5 mg in 10 µl) was mixed with 0.1 ml of glacial acetic acid. To this solution was added 0.4 ml of 0.1% lead tetraacetate (freshly recrystallized) in glacial acetic acid, and the reaction mixture was kept in the dark at room temperature. An aliquot (50 µl) was taken in an interval and mixed with 50 µl of 2% oxalic acid. The supernatant thus obtained was analyzed for pentose by the method of Brown (1946). The data expressed as per cent formation from mannose.

Fig. 3. Paper chromatogram of the partial acid hydrolysate of Olig. I-b.

A portion (1 mg) of Olig. I-b was hydrolyzed with 2 ml of 0.1 N oxalic acid for 4 h in a boiling water bath. After removal of oxalic acid with calcium carbonate, the hydrolysate was concentrated and applied on paper.

Paper, Toyo filter paper No.3; Solvent, ethylacetate-acetic acid-water (2:1:2, v/v, upper layer, kept for about 1 year); Staining reagent, alkaline-silver; Temperature, room temperature; Duration of irrigation, 12 h.

References: a, glycerol; b. N-acetylglucosamine; c, mannose; d, Olig. I-a; e, Olig. I-b.

TABLE I

Analytical date[1] of Olig. I-a and Olig. I-b before and after reduction with sodium borohydride[2]

	Olig. I-a		Olig. I-b	
	Before reduction	After reduction	Before reduction	After reduction
N-Acetylglucosamine(%)[3]	57.0(57.7)[6]	56.5	46.0(48.3)[7]	46.7
Mannose(%)[4]	47.0(47.0)[6]	1.4	40.0(39.4)[7]	39.8
Glycerol(%)[5]	0		21.9(20.1)[7]	21.4
$[\alpha]_D^{20}$ (Degree)	-11.6		+22.3	

1) Expressed as free compound.
2) An aqueous solution (0.5 mg in 3 ml) of the sample was reduced with 2 mg sodium borohydride for 15 h at 4°. Subsequently, the desalted solution was analyzed for its components.
3) Glucosamine was determined by the procedure of Yosizawa (1961).
4) Determined by the method of Masamune and Sakamoto (1956).
5) Calculated from the ratio of glycerol to mannose obtained by gas-chromatography: Gas-chromatography of the trimethylsilyl derivatives of these compounds was carried out by the procedure of Yamakawa and Ueta (1964), using a 3 m column packed with 5% Ucon-LB550 on Gas-chrom CLH at 205°.
6) Theoretical value for O-(2-acetamido-2-deoxy-D-glucopyranosyl)-D-mannose.
7) Theoretical value for O-(2-acetamido-2-deoxy-D-glucopyranosyl)-O-D-mannopyranosyl-glycerol.

showing its location at the reducing end. On the other hand, no reducing component was detected in Olig. I-b.

Oxidation of Olig. I-a with lead tetraacetate (Fig. 2), resulted in the quantitative formation of pentose from mannose molecule, indicating that the mannose residue was substituted at C_3 with N-acetylglucosamine. Levorotation of Olig. I-a (Table I) suggested the β-anomeric linkage between these monosaccharides.

Oxidation of Olig. I-b with 0.05 M sodium metaperiodate in

0.05 M acetate buffer (pH 4.5) in the dark for 24 h at 4°, resulted in the complete degradation of N-acetylglucosamine without any noticeable loss of the other components. This observation indicated that the N-acetylglucosamine residue was present at the non-reducing end and linked at C_3 of the mannose residue, and also that glycerol was substituted at C_2 with the mannose molecule. Furthermore, partial hydrolysis of Olig. I-b, with 0.1 N oxalic acid, produced glycerol, N-acetylglucosamine, mannose, and Olig. I-a (Fig. 3), indicating Olig. I-b was consisted of Olig. I-a and glycerol. Dextrorotation of Olig. I-b (Table I) suggested the α-anomeric configuration of the mannosyl linkage.

Oligs. II and III were also found to contain hexose and glucosamine. However, no further characterization was yet carried out.

Discussion----As reported previously (Sato et al., 1967), 38% of mannose residues and 70% of glucosamine residues of the hydrazinolysate of the α_1-acid glycoprotein were oxidized with periodate. Moreover, hydrolysis with 1 N sulfuric acid (4 h, 100°) seemed to cleave most of the glycosidic linkages in the material, affording only small amounts of oligosaccharides. Glycerol found in Olig. I-b must be the product derived from the monosaccharide adjacent to mannose by the oxidation and reduction. The present results provided an evidence for the presence of 0-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-0-α-D-mannopyranosyl structure in the inner core of the carbohydrate moiety of the α_1-acid glycoprotein as proposed previously by the present authors (Sato et al., 1967).

Summary----Hydrazinolysate of the α_1-acid glycoprotein of human plasma was oxidized with sodium metaperiodate and subsequently reduced with sodium borohydride. After N-acetylation of the

acid hydrolysate of the above product, several oligosaccharides were isolated. Two of them were characterized to be O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-D-mannose and O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-O-α-D-mannopyranosyl-(1→2)-glycerol.

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