A STRUCTURAL INVESTIGATION OF A D-GLUCAN FROM *Yersinia pseudotuberculosis* OF SEROTYPE VI

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It has been established that an α-D-glucan isolated from *Yersinia pseudotuberculosis*, serotype VI, is branched and contains α-1→4- and α-1→6-bound D-glucopyranose residues in the main chain and the side chains, respectively.

Glucans form a broad and diverse class of polysaccharides. They are one of the component parts of the cell wall and play the role of reserve polysaccharides. The most common are branched glucans with α-1,4-bonds. It has been established that glucans possess biological activity [1].

A D-glucan was isolated simultaneously with a lipopolysaccharide (LPS) from *Yersinia pseudotuberculosis* of serotype VI [2] by the extraction of the bacterial cells with 45% aqueous phenol. The LPS was separated by three ultracentrifugations of the aqueous fraction. For the complete separation of the glucan from the LPS in the supernatant liquid we used ultrafiltration followed by the digestion of the nucleic acids with nucleases, the separation of the protein by Sevag's method, and the isolation of the glucan by gel filtration on Sephadex G-100, where it issued after the free volume of the column.

The glucan isolated is most probably a reserve polysaccharide which accumulates in microorganisms on aging and when they are transferred onto artificial media under laboratory conditions. Only D-glucose was detected in a hydrolysate of the glucan. The high positive angle of rotation of the glucan, $[\alpha]_{578}^{20}$ +135° (c 0.45; water) shows that the glucose residues are connected by α-bonds. The gas–liquid chromatography of the acetates of the partially methylated methyl glycosides obtained by methanolysis of the completely methylated glucan showed the presence of 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-O-methyl-D-glucoses in a ratio of 1:2:1. The presence of 2,3,6-tri-O-methyl-D-glucose shows that in the main chain of the glucan the D-glucopyranose residues are bound by 1,4-glucosidic bonds, and the identification of 2,3-di-O-methyl-D-glucose shows the presences of branchings at C-6 in the D-glucopyranose residues of the main carbohydrate chain.

The results of methylation were also confirmed by ^{13}C NMR spectroscopy. In the ^{13}C NMR spectrum of the glucan (Fig. 1) two signals are observed in the region of the resonance of the anomeric carbon atoms at 100.6 and 99.05 ppm, with an integral ratio of 3:1. Such values of the chemical shifts (CSs) of the signals of the anomeric carbon atoms are characteristic for the α configuration of the glycosidic bonds in glucans [3]. The α configuration of the glycosidic bonds is also confirmed by analysis of the 75–80 ppm region, where signals of only the ring carbon atoms participating in the formation of glycosidic bonds are observed and not the signals of the C-5 atoms characteristic for the β configuration [3].

A comparison of the values of the ^{13}C CSs in the spectrum of the glucan under investigation with the ^{13}C spectra of disaccharides (maltose and isomaltose) shows that the CSs of the carbon atoms of the glucan correspond with those of similar atoms for α-1→4- and α-1→6-bound glucobioses [4], which enables the signals of the carbon atoms in the ^{13}C spectrum of the polymer to be assigned.

The signals at 100.6, 78.8, and 61.5 ppm relate, respectively, to the C-1, C-4, and C-6 atoms of the α-1→4-bound monosaccharide units, and signals at 99.05 and 68.5 ppm to the C-1 and C-6 atoms of monosaccharide residues bound by α-1→6-glycosidic bonds. The ratio of the integral intensities of the C-atoms in the ^{13}C spectrum permits the assumption that of the possible alternatives the most probably structure of the glucan is that shown in Fig. 2.

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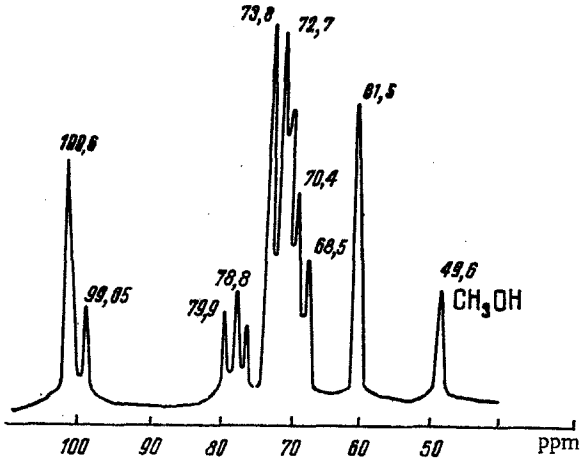

Fig. 1. ^{13}NMR spectrum of the glucan from *Yersinia pseudotuberculosis* of serotype VI.

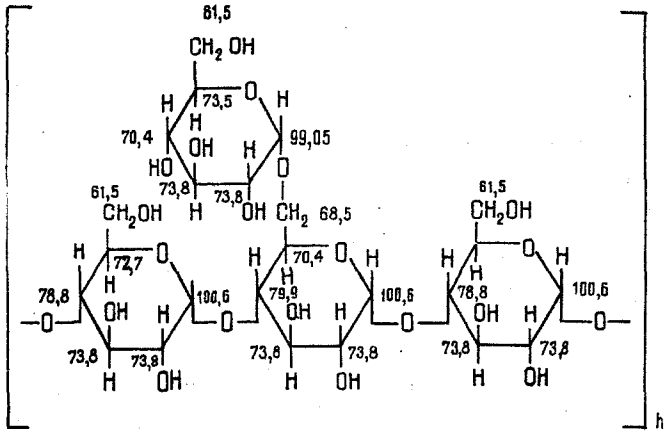

Fig. 2. Structure of the glucan from *Yersinia pseudotuberculosis* of serotype VI.

EXPERIMENTAL

Decending paper chromatography was performed on Filtrak FN-3 paper in the solvent system butan-1-ol–pyridine–water (6:4:3 by volume). Monosaccharides were denoted with an alkaline solution of silver nitrate and with aniline hydrogen phthalate. Gas–liquid chromatography was performed on a Pye-Unicam 104 chromatograph with a flame-ionization detector using glass columns (150 × 0.4 cm) containing 3% of QF-1 Gas-Chrom Q (100-200 mesh). Monosaccharides were analyzed in the form of the acetates of the corresponding polyols and the acetates of the methyl glycosides at temperatures of 175-225 and 120-225°C, the rate of change of temperature being 5 deg/min. The rates of flow of argon and hydrogen were 60 ml/min.

Chromato-mass spectrometry was carried out on an LKB-9000 instrument using a column with the QF-1 phase. IR spectra were recorded on a UR-20 spectrophotometer in chloroform.

The ^{13}C NMR spectrum was obtained on a Bruker-Physik HX-90E instrument at 22.63 MHz in D_2O solution at 80°C. Methanol was used as internal standard. The chemical shifts are given in parts per million after recalculation by means of the relation $\delta_{TMS} = \delta_{MeOH} + 49.6$ ppm. Optical rotation were measured on a Perkin-Elmer 141 polarimeter.

Microorganisms. A strain of *Y. pseudotuberculosis* of serotype VI was obtained from Dr. M. Tsubakura (Japan). The organisms were grown on a synthetic medium [5].

Isolation of the Glucan. The dry cells (80 g) were extracted with 45% aqueous phenol [6]. The aqueous extract was thrice ultracentrifuged at 105,000g. The supernatant liquid was ultrafiltered through a Ripor 2-64 membrane under a pressure of 2 atm. The solution

that had passed through the membrane was concentrated to 10 ml and was precipitated with 4 volumes of ethanol. The precipitate was dissolved in 10 ml of phosphate buffer, pH 7, and then 1 mg of ribonuclease (Koch-Light Laboratories) was added and the mixture was kept at 37°C for 12 h.

The solution was brought to pH 9 with alkali, 1 mg of deoxyribonuclease (Koch-Light Laboratories) and 0.003 g-mole of magnesium sulfate were added, and incubation was carried out at 37°C for 7 h. The nucleases were deactivated by boiling for 5 min. The protein was separated by Sevag's method [7]. The solution was dialyzed for 2 days against distilled water, evaporated, and precipitated with ethanol. The precipitate was chromatographed on Sephadex G-100 using a 100 × 3 mm column. The fractions were determined by the phenol—sulfuric acid method. The glucan issued in the first peak after the free volume of the column. The yield of glucan was 10 mg.

Methylation. The glucan was methylated by Hakomori's method [8]: To 5 mg of the glucan were added 0.5 ml of dimethyl sulfoxide and 0.5 ml of methylsulfinyl carbanion. After 8 h, 1 ml of methyl iodide was added to the mixture and it was stirred for 12 h. The methylated glucan was extracted with chloroform. The yield of methylated glucan was 4 mg. The completeness of methylation was checked by the absence of absorption bands of hydroxy groups (3400-3600 cm^{-1}) in the IR spectrum.

The methylated glucan (2 mg) was heated with 1 ml of methanolysis mixture ($HClO_4$—methanol (1:10)) at 100°C for 3 h, neutralized with Dowex-1 (HCO_3^-), evaporated, and acetylated. The methyl esters were identified with the aid of mass spectrometry and comparison with authentic samples.

SUMMARY

An α-D-glucan has been isolated from the pseudotuberculosis microbe *Yarsinia pseudotuberculosis* of serotype VI. It has been shown with the aid of methylation and ^{13}C NMR spectroscopy that the glucan is branched and contains α-1→4-D-glucopyranose residues in the main chain and α-1→6-D-glucopyranose residues in the side chains.

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