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STUDIES OF THE POLYSACCHARIDE MOIETY OF CORALLAN, A GLYCOPROTEIN FROM *Pseudopterogorgia americana*

VALENTINA I. MOLCHANOVA, RAISA G. OVODOVA, YURY S. OVODOV, YURY N. ELKIN,
Pacific Institute of Bioorganic Chemistry, Far East Science Centre, Academy of Sciences of the U.S.S.R., Vladivostok, 690022 (U.S.S.R.)

AND VIOLETA FERNANDEZ SANTANA
National Institute of Oncology and Radiobiology, Havana (Cuba)

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ABSTRACT

The carbohydrate chains of a glycoprotein, corallan, from the Caribbean soft-coral *Pseudopterogorgia americana*, contain D-glucose, D- and L-galactose, D-arabinose, L-fucose, and D-glucuronic acid. Methylation studies, partial acid hydrolysis, and Smith degradation have been used to elucidate some structural features of the polysaccharide moiety.

INTRODUCTION

Corallan, isolated from the widespread, Caribbean soft-coral *P. americana*, is a sulphated glycoprotein[1] and we now report some structural features of the polysaccharide moiety.

RESULTS AND DISCUSSION

Corallan was desulphated by acidic methanolysis[2] and solvolysis[3]. The former method afforded a product (desulphated corallan, DSC) which contained 1% of sulphate. Acid hydrolysis of DSC gave D-arabinose, D- and L-galactose, L-fucose, D-glucose, and D-glucuronic acid in the molar ratios 7:6:5:1:1, which are similar to those of the sugars in the parent corallan. However, methanolysis causes loss of some of the D-arabinose residues, thus indicating their terminal position.

DSC was subjected to two methylations (Hakomori[4]). Hydrolysis of the methylated polymer afforded 2,3,4,6-tetra-, 2,3,4- and 3,4,6-tri-, and 2,4- (trace) and 4,6-di-*O*-methylgalactose; 2,3,6-tri-, 4-, and 6-*O*-methylglucose; 2,3,4-tri- and 2,4-di-*O*-methylarabinose; and 2,3,4-tri- and 3,4-di-*O*-methylfucose; which were identified by g.l.c.–m.s. The formation of fully methylated derivatives of arabinose, fucose, and galactose indicated that corallan has a branched structure and that these sugar residues occupy terminal positions. The formation of 3,4-di-*O*-methylfucose

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and 2,4-di-*O*-methylarabinose indicated that these residues are involved in (1→2) and (1→3) linkages, respectively. Likewise, the galactose residues are involved in (1→2) and (1→6) linkages. The formation of di- and mono-*O*-methylhexose derivatives reflects the presence of branch points or the location of sulphate groups lost during hydrolysis of methylated DSC. The methylation data also demonstrated that all the sugar residues in corallan are pyranoid.

DSC reduced 0.55 mol of periodate per "anhydro sugar" unit, reflecting the presence of (1→3) linkages, branches, or sulphate groups, which prevented oxidation of sugar residues. The last possibility is emphasised by the fact that corallan reduced only 0.39 mol of periodate. The polyaldehyde obtained from DSC was reduced with sodium borohydride, and hydrolysis of the resulting polyalcohol gave arabinose, fucose, galactose, glucose, and glycerol in the molar ratios 1:1:4:2:2. The content of uronic acid was 5.6%. The formation of glycerol demonstrated the presence of (1→6)-linked residues of galactose or glucose, thus confirming the findings of the methylation analysis. The low contents of arabinose and fucose in the polyalcohol reflected the terminal positions of these sugars in the polysaccharide moiety of corallan. The increased contents of glucose and galactose in the polyalcohol, in comparison with those in corallan, demonstrated the presence of sulphate groups on these residues or their involvement in branching. Hydrolysis of the polyalcohol with dilute hydrochloric acid and chromatography of the products on Sephadex G-15 afforded a degraded polysaccharide and oligosaccharide fractions. The former contained the same sugar residues as corallan but in different proportions. Preparative p.c. of the oligosaccharide fractions gave maltose (R_{Gal} 0.81, 4.6 mg) and maltotriose (R_{Gal} 0.55, 4.3 mg), the structures of which were established by methylation analysis.

When corallan was treated with dilute sulphuric acid, arabinose and fucose could be detected in the hydrolysate after 5 min, galactose after 15 min, and glucuronic acid after 1.5 h. This sequence reflected the terminal positions of arabinose and fucose in the polysaccharide. Partial hydrolysis of corallan with acid afforded a complex mixture of mono- and oligo-saccharides, which was subjected to chromatography on Bio-Gel P-2 followed by preparative p.c.

On the basis of specific optical rotations, the monosaccharides were identified as D-glucose, D-arabinose, D-glucuronic acid, L-fucose, and a mixture of D- and L-galactose. The oligosaccharides **3–6** were also obtained.

Oligosaccharide **3** had $[\alpha]_D^{20}$ +39°, R_{Gal} 0.3, and gave galactose and arabinose (3:1) on hydrolysis. Methylation analysis indicated the structure D-Ara*p*-(1→2)-Gal*p*-(1→2)-Gal*p*-(1→2)-Gal*p*.

Oligosaccharide **4** had $[\alpha]_D^{20}$ +60°, R_{Gal} 0.51, and contained galactose and fucose in the ratio 2:1, with fucose at the reducing end. Methylation analysis indicated the structure Gal*p*-(1→2)-Gal*p*-(1→2)-L-Fuc.

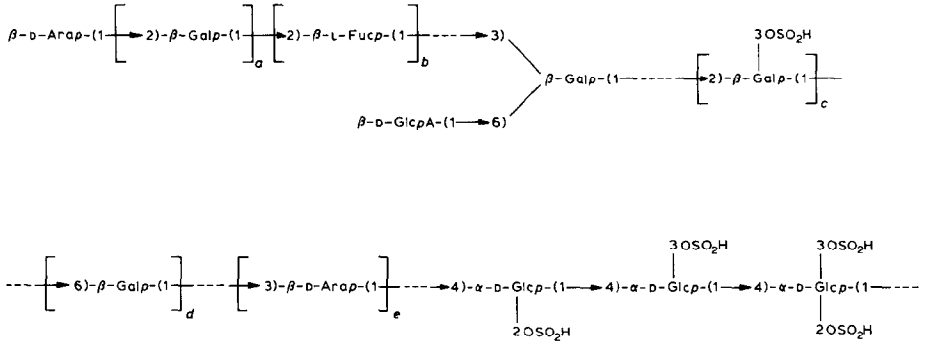
Oligosaccharide **5** had $[\alpha]_D^{20}$ +12°, R_{Gal} 0.5, and contained galactose only. Methylation analysis indicated the structure Gal*p*-(1→2)-Gal*p*-(1→2)-Gal. The $[\alpha]_D$ value of **5** suggested the presence of β linkages.

Oligosaccharide **6** had $[\alpha]_D^{20}$ −33°, R_{GalA} 0.77, and contained galactose and glucuronic acid (1:1), with the former at the reducing end. Hydrolysis of methylated **6** gave methylated glucuronic acid and 2,3,4-tri-*O*-methylgalactose. The $[\alpha]_D$ value of **6** indicated the glycosidic bond to be β, and hence the structure to be β-D-Gal*p*A-(1→6)-Gal.

Due to its higher solubility, the Cetavlon salt of corallan was subjected to acetolysis. Preparative p.c. of the deacetylated products gave arabinose, fucose, galactose, and oligosaccharides **5** and **6**.

Oxidation of acetylated corallan with chromic anhydride[5] destroyed the arabinose, fucose, galactose, and glucuronic acid residues, indicating that they were β-linked and that the glucose residues were α-linked.

Thus, it is concluded that the polysaccharide moiety of corallan contains the following structural features, in addition to terminal D-Ara, Gal, and L-Fuc groups:



The locations of some of the D-arabinose, L-fucose, and galactose residues and sulphate groups remain to be determined. Investigation of the structure of corallan is continuing.

EXPERIMENTAL

General methods. — Solutions were concentrated at <40° under reduced pressure. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. P.c. was carried out on Filtrak FN-3 and FN-15 and Whatman 3MM papers, using 1-butanol–pyridine–water (6:4:3), 1-butanol–acetic acid–water (4:1:5, upper layer), and ethyl acetate–pyridine–acetic acid–water (1:1:1:1).

G.l.c. was performed with a Pye Unicam gas chromatograph equipped with a flame-ionisation detector and glass columns (200 × 0.4 cm) containing 3% of QF-1 on Chromosorb Gas Chrom Q (100–120 mesh) with temperature programmes of 175→225° (alditol acetates) and 125→225° (methyl glycoside acetates[6]) at 4°/min and an argon flow rate of 33 mL/min. G.l.c.–m.s. was performed with an LKB-9000 instrument. I.r. spectra were recorded with a UR-20 spectrophotometer.

Corallan was extracted from the Caribbean soft-coral *Pseudopterogorgia americana* harvested from the sublittoral of Cuba island.

Desulphation of corallan. — (*a*) *Methanolysis.* A suspension of corallan (300 mg, dried *in vacuo* over P_2O_5) in dry methanolic 0.1M HCl (30 mL) was vigorously stirred at 20° for 16 h and then centrifuged. The procedure was thrice repeated. A solution of the final precipitate in water was neutralised with sodium carbonate, dialysed, and lyophilised to yield a desulphated corallan (DSC, 254 mg). When the methanolic solutions were combined, neutralised, and concentrated, and the residue was subjected to p.c., arabinose was detected.

(*b*) *Solvolysis.* A solution of the sodium salt of corallan (400 mg) in water (400 mL) was stirred with KU-2(H^+) resin, filtered, neutralised with pyridine, concentrated, and lyophilised to furnish the pyridinium salt of corallan (372.5 mg). This salt was mixed with dry 1,4-dioxane (12.5 mL) containing 2% of pyridine, and the mixture was kept for 10 h at 70°, dialysed, and lyophilised to furnish partially desulphated corallan (3.6% sulphate). Repetition of the above procedure gave material (287 mg) containing 3.2% of sulphate.

Acid hydrolyses. — (*a*) Corallan or DSC (10–15 mg) was treated with M sulphuric acid (1 mL) at 100° for 8–10 h. Each mixture was neutralised ($BaCO_3$) and concentrated, and the residue was subjected to p.c. and g.l.c. (after reduction with borohydride and acetylation).

(*b*) A solution of corallan (100 mg) in 25mM H_2SO_4 (100 mL) was kept at 100° for 1 h, neutralised ($BaCO_3$), filtered, concentrated, and poured into ethanol (4 vol.). The precipitate was removed by centrifugation, and the supernatant solution was concentrated. The residue (65 mg) was eluted from a column (2.5 × 70 cm) of Bio-Gel P-2 with water. The fractions (5 mL) were analysed by p.c., combined as appropriate, and concentrated to afford D-arabinose (3.5 mg), $[\alpha]_D^{20}$ −168° (water); L-fucose (2.5 mg), $[\alpha]_D^{20}$ −122° (water); D- + L-galactose (2.8 mg), $[\alpha]_D^{20}$ +17° (water); D-glucose (2.1 mg), $[\alpha]_D^{20}$ +108.5° (water); and oligosaccharides **3** (3.7 mg), **4** (4.2 mg), **5** (4.2 mg), and **6** (17 mg).

Acetolysis of corallan. — The Cetavlon salt of corallan (180 mg, dried *in vacuo* over P_2O_5) was added at 5° to a mixture of acetic anhydride (6 mL), acetic acid (4 mL), and conc. sulphuric acid (0.6 mL). The mixture was vigorously stirred for 45 h at 20° and then centrifuged, and the supernatant solution was poured into ice–water. The mixture was neutralised ($NaHCO_3$) to pH 6.0 and extracted with chloroform (3 × 5 mL). The combined extracts were dried (Na_2SO_4) and concentrated. The residue (137 mg) was dried over P_2O_5 and to a solution in dry methanol (4 mL) at 5° was added methanolic 2M sodium methoxide (0.5 mL). The mixture was kept for 16 h at 4°, neutralised with KU-2(H^+) resin, filtered, and concentrated. Preparative p.c. of the residue (105 mg) yield D-arabinose, L-fucose, D- + L-galactose, and oligosaccharides **5** (3.7 mg) and **6** (4.3 mg).

Methylation analysis. — A solution of corallan (30 mg, dried over P_2O_5) in dry methyl sulphoxide (1 mL) was methylated twice (Hakomori[4]) to afford methylated corallan (27.2 mg). Methylated corallan (10 mg) was treated with dry methanolic M HCl (1.5 mL) in a sealed tube for 6 h at 100°. The methanolysate was concentrated, the residue was treated conventionally with acetic anhydride (0.2

mL) in pyridine (0.2 mL), and the acetylated methyl glycosides were subjected to g.l.c. and g.l.c.–m.s.

Smith degradation. — A solution of corallan (100 mg) in water (50 mL) was mixed with 0.03M sodium metaperiodate (50 mL) and kept in the dark at 4°. The reduction of periodate was determined spectrophotometrically at 220 nm. After 24 h, ethylene glycol (1 mL) was added to the mixture which, after 1 h, was then dialysed and treated with an excess of sodium borohydride for 15 h. The excess of borohydride was destroyed with acetic acid, and the mixture was dialysed and then lyophilised to furnish the polyalcohol (57 mg). A portion (10 mg) was heated with M sulphuric acid for 8 h at 100°, the mixture was neutralised ($BaCO_3$) and centrifuged, and the supernatant solution was concentrated. P.c. of the residue revealed glycerol, arabinose, fucose, galactose, and glucuronic acid. A solution of the polyalcohol (50 mg) in 0.25M HCl (50 mL) was kept for 16 h at 20°, neutralised, concentrated, and eluted from a column (1.5 × 60 cm) of Sephadex G-15 with water to afford fractions of high (37 mg) and low molecular weight (9.9 mg). Preparative p.c. of the latter on Whatman 3MM paper gave maltose (4.6 mg) and maltotriose (4.3 mg).

Hydrolysis of the fraction of higher molecular weight afforded all the constituents of the polyalcohol.

Oxidation of corallan with chromic anhydride. — A solution of corallan (200 mg, dried over P_2O_5) in formamide (3 mL) was acetylated thrice with acetic anhydride (8 mL) in pyridine (2 mL), in the usual manner. The acetylated corallan (17 mg) had $[\alpha]_D^{20}$ −61° (water) (acetyl, 21%). A mixture of corallan acetate (5 mg), chromic anhydride (25 mg), and glacial acetic acid (0.5 mL) was kept for 2 h at 50°, neutralised with Dowex 1-X4 (HCO_3^-) resin, and concentrated. The residue was hydrolysed with M sulphuric acid for 4 h at 100°. Glucose was identified (p.c.) in the hydrolysate.

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