Galactosamine Glycan of Chondrococcus columnaris

Abstract. *The marked viscosity of liquid cultures of the myxobacterium* Chondrococcus columnaris *is caused by production of an extracellular polysaccharide. The polysaccharide is a high-molecular-weight homopolymer of* D-*galactosamine in which the galactosamine subunits are connected by* α-*(1→4) glycosidic linkages. Half of the amino groups are acetylated.*

Microorganisms produce a variety of extracellular polysaccharides (*1*). The myxobacteria were so named because of their slimy appearance, but the extracellular secretions responsible have not been extensively studied. Holt (*2*) extracted extracellular slime from *Myxococcus xanthus* and found it to contain protein, polysaccharide, lipid, and nucleic acid. The polysaccharide fraction contained pentoses, hexoses, hexuronic acids, and the amino sugars —glucosamine and galactosamine. Ordal (*3*) observed that liquid cultures of the fish pathogen *Chondrococcus columnaris* may become very viscous and that colonies stain with simple acid dyes such as Congo red, though individual cells do not take up the negative stains. Colonies of *Chondrococcus coralloides* and *Myxococcus* sp. also stain with Congo red, but several nonfruiting myxobacteria tested failed to take up the dye. We now describe the chemical nature of a Congo red–staining material produced by *C. columnaris*.

Chondrococcus columnaris, strain RR-1A, was grown in a medium of mineral salts and casein hydrolyzate (CM medium, *4*) containing 0.1 percent glucose. Cultures were incubated at room temperature on a reciprocating shaker. The cells were removed from the culture fluid by centrifugation for 1 hour at 25,000 rev/min. The supernatant was a nonturbid, viscous solution containing about 200 mg of polysaccharide per liter. The polysaccharide could be removed from solution by adding two volumes of ethanol and spooling the precipitate onto a glass rod. Excess ethanol was removed from the gelatinous precipitate by pressing the glass rod against the side of the beaker. After the gummy precipitate adhering to the glass rod was partially dried in air, it was placed in a small volume of distilled water, and the rod was agitated until the polysaccharide was freed. This preparation was then dialyzed against distilled water for 48 hours at 3°C; the dialyzed material was diluted with distilled water and shaken until a homogeneous solution was obtained. The resulting colorless solution was extremely viscous and contained about 300 mg of polysaccharide per liter. In order to obtain more concentrated solutions, it was necessary to reduce the viscosity by shearing with a French pressure cell or by treatment with high-frequency sound before removal of solvent.

The polysaccharide was obtained as fluffy white solid by lyophilization of dilute homogeneous solutions. The lyophilized polysaccharide dissolved only very slowly and incompletely in water; it dissolved more rapidly in dilute hydrochloric acid without noticeable loss of viscosity. It is dextrorotatory, $[\alpha]^{25}_{D}$ + 140° (0.3 percent, water), has the empirical formula $C_{14}H_{24}N_2O_9$, and gives a weak ninhydrin test. The carbazole test for uronic acids (*5*) and the Elson-Morgan test for 2-aminohexoses (*6*) were negative. Amino acids were not detected in hydrolyzates.

Heating the polysaccharide in hydrochloric acid rapidly destroyed its viscosity. Paper chromatography of acid hydrolyzates revealed a single product of hydrolysis, an amino sugar, which gave positive tests with ninhydrin, Tollens reagent, aniline hydrogen phthalate, and the Elson-Morgan reagents. Lack of discoloration during the hydrolysis indicated absence of any simple pentose or hexose in the polysaccharide.

The amino sugar was crystallized directly from the hydrolyzate in good yield, a further indication of the homogeneity. It was identified as D-galactosamine hydrochloride by its decomposition point, 175° to 180°C, specific rotation (final value $[\alpha]^{25}_{D}$ + 88°), by nuclear magnetic resonance spectrum, and by chromatographic comparison of both the hydrochloride and the *N*-acetyl derivative with authentic galactosamine and glucosamine derivatives in several solvent systems (Table 1) including borate buffer. This buffer separates the *cis*-glycol, *N*-acetyl-D-galactosamine, from its *trans*-epimer, *N*-acetyl-D-glucosamine (*7*). Deamination of the amino sugar with ninhydrin (*8*) gave a pentose chromatographically identical with D-lyxose obtained by deamination of authentic D-galactosamine and different from D-arabinose obtained by deamination of D-glucosamine.

These findings establish the structure of the polysaccharide as a galactosaminoglycan, a homopolymer of galactosamine. The anomeric centers are assigned to the α-configuration on the basis of Hudson's isorotation rules (*9*). Elemental analysis indicated the presence of acetyl groups in one-half of the galactosamine units. The presence of acetyl groups was confirmed in the computer-averaged nuclear magnetic resonance spectrum of polysaccharide which had been treated briefly with hot hydrochloric acid to reduce viscosity. The acetyl groups were shown to be *N*-acetyls rather than *O*-acetyls by an infrared absorption band at 1640 cm^{-1} and by the absence of absorption in the vicinity of 1730 cm^{-1}.

The galactosaminoglycan was oxidized rapidly by sodium metaperiodate. The oxidation, followed titrimetrically

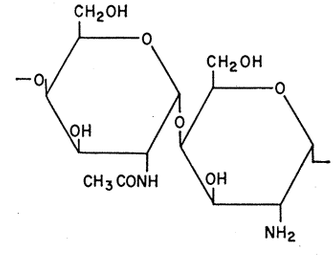

Fig. 1. Extracellular polysaccharide of *Chondrococcus columnaris*.

Table 1. Chromatographic comparison of amino sugar from *Chondrococcus* polysaccharide with glucosamine (the standard) and galactosamine.

Derivative	Glucosamine (R_{rel})	Galactosamine (R_{rel})	Amino sugar (R_{rel})
Hydrochloride*	1.00	0.91	0.89, 0.90
Hydrochloride†	1.00	.84, 0.84	.83, .85, 0.85
N-acetyl‡	1.00	.63, .63	.61, .62
Pentose (after deamination)*	1.19, 1.20§	1.34, 1.34	1.34, 1.34

* Solvent system consisting of *t*-butyl alcohol, acetic acid, and water (2 : 1 : 1). † Mixture of ethyl acetate, pyridine, and water (8 : 2 : 1). ‡ Mixture of *n*-butyl alcohol, pyridine, and water (6 : 4 : 3) on borate-impregnated paper. § Authentic D-arabinose had R_{rel} 1.20, 1.20.

and polarimetrically, was complete within 4 minutes at 5°C, with consumption of 0.6 mole of periodate per galactosamine unit. No further consumption of periodate or change in optical rotation was observed after 3 days. After 1 week in excess periodate the oxidized polysaccharide was recovered by dialysis and lyophilization. The oxidized product did not react with ninhydrin. A significant quantity of galactosamine was identified in the hydrolyzate of the periodate-oxidized polysaccharide.

If the polysaccharide is a simple linear polymer, only an α-(1→4)-glycosidic link between galactosamine units (Fig. 1) is consistent with the observation that a half mole of periodate is consumed per galactosamine residue and a significant amount (half) of the galactosamine is intact after oxidation. The periodate consumed destroys that half of the galactosamine units that are unacetylated by cleavage between carbons No. 2 and No. 3, while *N*-acetylation protects the remainder of the galactosamine moieties from oxidation. An alternating (1→3)-(1→4)-linked galactosaminoglycan in which the (1→3)-linked units are *N*-acetylated would also consume half a mole of periodate in destruction of the *N*-acetylgalactosamine moieties; the unacetylated galactosamine would survive. Such an alternating (1→3), (1→4) pattern occurs in the glucan nigerose (*10*). However, this more complicated structure is ruled out by the observation that periodate-oxidized polymer does not react with ninhydrin, which indicates that the amino groups of surviving galactosamine units are not free but masked by acetyl groups.

Whether the acetylated and unacetylated galactosamine units are ordered in some repeating pattern or whether the polysaccharide results from random acetylation of α-(1→4)-galactosaminoglycan is not yet known. A galactosaminoglycan consisting solely of galactosamine residues which are approximately one-third *N*-acetylated has been isolated from *Aspergillus parasiticus* (*11*). The occurrence of a galactosamine polymer which is 38-percent acetylated in *A. parasiticus* and 50-percent acetylated in *Chondrococcus columnaris* might suggest incomplete and random acetylation of the same simple galactosaminoglycan. However, the galactosaminoglycan from *A. parasiticus* has a much lower reported specific rotation, $[\alpha]^{25}_{D}$ + 51.5°, than that of the *Chondrococcus* glycan, suggesting the presence of comparable amounts of α- and β-glycosidic links in the *Aspergillus* glycan. Since these two *N*-acetylated galactosaminoglycans seem to differ in backbone linkage, it is unlikely that they are examples of a single randomly acetylated aminopolysaccharide.

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Inhibition of Insulin Release by Norepinephrine in Man

Abstract. *Normal subjects were given glucose (300 mg/min) or tolbutamide (1 g, intravenously), alone and during intravenous infusions of norepinephrine (6 μg/min). Immunoreactive insulin concentration was less than expected during the infusions of norepinephrine, but returned to higher values after the norepinephrine infusions. From these data it is concluded that norepinephrine inhibits the release of insulin from pancreatic beta cells.*

Epinephrine infusions in man inhibit the expected rise in peripheral immunoreactive insulin (IRI) when given alone or simultaneously with glucose, glucagon, or tolbutamide (*1, 2*). These observations, together with data obtained with slices of pancreas from rabbits (*3*) and intact dogs (*4*), suggest that epinephrine acts directly on the pancreatic cell to block the release of insulin.

The physiological role of epinephrine may be restricted to its relatively infrequent discharge from the adrenal medulla; but norepinephrine, as the neurochemical transmitter (*5*), would appear to be secreted continuously as the final effector of the sympathetic nervous system. Therefore, it was of interest to test whether norepinephrine inhibited the release of IRI, as has been demonstrated for epinephrine.

The subjects were healthy young men and women 21 to 31 years of age, who were within 15 percent of their ideal body weight and had no known blood relatives with diabetes mellitus. They were maintained at rest in a supine position during the test interval. They were fasted overnight (14 hours), and then in each one an indwelling plastic cannula was inserted into an antecubital vein in one arm for blood sampling, and a scalp vein needle was inserted in a hand vein of the other arm for drug infusions. A slow infusion of 0.85 percent NaCl was given, to prevent clotting, through each catheter throughout the 3-hour period of observation. Blood samples were taken for analysis of glucose, free fatty acids, and insulin at 15-minute intervals. A mean of four values taken during the first 1-hour control period was set to 100 percent, and all these and subsequent values are expressed as a percentage of the mean control value.

Heparinized plasma was kept at 0°C, centrifuged in the cold, and frozen at −19°C until analyzed for free fatty acids (*6*), and glucose (*7, 8*). Whole blood was kept at 0°C until the sampling was finished, allowed to clot for 1 hour at room temperature, and centrifuged at 0°C. This serum was then frozen at −19°C until analyzed for IRI by the double-antibody immunoprecipitation technique (*9*). Each subject received intravenously either norepinephrine (6 μg/min) (Levophed bitartrate), in 0.85 percent NaCl for 1 hour, or tolbutamide (1 g) (Orinase) injected within 1 minute as its sodium salt in 20 ml of water.

An infusion (6 μg/min.) of norepinephrine raised plasma FFA markedly and glucose slightly (Fig. 1), but had no effect on serum IRI until after the infusion was stopped, when a small rise in IRI was noted. With this small hyperglycemic response it was impossible to judge whether an increase in IRI during the norepinephrine infu-