JOURNAL OF BACTERIOLOGY, Feb. 1980, p. 971-972
0021-9193/80/02-0971/02$02.00/0 Vol. 141, No. 2

Primary Structure of the *Escherichia coli* Serotype K30 Capsular Polysaccharide

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Methylation, ^{1}H nuclear magnetic resonance, and bacteriophage degradation results indicate that the *Escherichia coli* serotype K30 capsular polysaccharide consists of →2)-α-D-Man*p*-(1→3)-β-D-Gal*p*-(1→ chains carrying β-D-GlcUA*p*-(1→3)-α-D-Gal*p*-(1→ branches at position 3 of the mannoses.

In the course of our studies on the substrate specificity of bacteriophage-borne (spike-associated) glycanases depolymerizing *Enterobacteriaceae* capsular (e.g., 9, 13) and cell wall polysaccharides (12), we have compared the oligosaccharides obtained by the action of *Klebsiella* bacteriophage no. 20 on the *Klebsiella* serotype K20 and *Escherichia coli* K30 capsular polysaccharides (13), since different primary structures (3, 5) have been reported for these two substrates of one viral enzyme. Indistinguishable split products, however, were obtained from both materials. Therefore, the *E. coli* K30 glycan was reanalyzed by methylation, gas-liquid chromatography, mass spectrometry, and ^{1}H nuclear magnetic resonance, which had not been used in the earlier study (5). It was found that the *E. coli* polysaccharide probably has the same structure as the *Klebsiella* K20 antigen (possible differences in *O*-acetyl substitution not considered).

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The K30 polysaccharide was extracted from *E. coli* E69 (O9:K30[A]:H12) (reference 5, method A) and subjected to the analytical procedures previously described or cited (10, 11). The material was found to consist of D-glucuronic acid, D-mannose, and D-galactose in a molar ratio approaching 1:1:2.

The results of methylation, gas-liquid chromatography, and mass spectrometry are summarized in Table 1. It can be deduced that the aldobiouronic acid is D-GlcUA*p*-(1→3)-D-Gal and that it constitutes branches at position 3 of the mannoses in a →2)-D-Man*p*-(1→3)-D-Gal*p*-(1→ chain.

TABLE 1. *Identification and ratios of O-acetyl-O-methylalditols obtained from E. coli serotype K30 capsular polysaccharide and its derivatives*

Peracetyl derivative of[a]:	T^b		Primary fragments found (m/e)						Ratio of peak integrals			
	Literal	Found	45	117	161	189	233	261	I[c]	II	III	IV
2,4,6-ManOH[d]	2.09	2.15[d]	+	+	+		+		–	–	–	1.0
2,4,6-GalOH	2.28	2.28	+	+	+		+		1.0	1.7	1.6	1.8
2,3,4-GlcOH	2.49	2.42		+	+	(191)[e]	(235)[e]		–	–	0.8	–
4,6-ManOH	3.29	3.38	+		+			+	–	1.0	1.0	–

[a] 2,4,6-ManOH = 2,4,6-Tri-*O*-methyl-D-mannitol, etc.

[b] *T*, Retention time, relative to peracetylated 2,3,4,6-GlcOH (T = 1.00) and 2,3-GlcOH (T = 5.39) in gas-liquid chromatography on ECNSS-M (1).

[c] I, Aldobiouronic acid, consisting of GlcUA and Gal, as obtained by partial acid hydrolysis of the polysaccharide (5), permethylated (the GlcUA derivative is not registered by the methods used); II, polysaccharide, permethylated; III, polysaccharide, permethylated and then carboxyl reduced/dideuterated; IV, repeating unit tetrasaccharide ending in reducing Gal, as obtained by bacteriophage degradation of the polysaccharide (see text), permethylated.

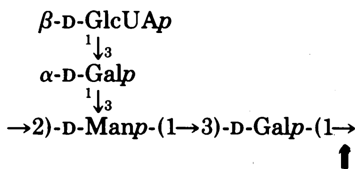
[d] Cochromatographing with standard.

[e] Dideuterated fragment found instead.

The proton magnetic resonance spectrum of the K30 glycan (1) showed, inter alia, four signals of about equal integrals at δ 4.57, 4.67, 5.20, and 5.35, indicating two β and two α linkages per repeating unit (due to the line width of the signals, coupling constants could not be determined).

Incubation (40 h at 37°C) of *E. coli* K30 or of *Klebsiella* K20 capsular polysaccharide (3.8 mg/

ml of phosphate-buffered physiological saline [pH 7.2] containing 0.05% sodium azide) with purified particles of *Klebsiella* bacteriophage no. 20 (1.3×10^{10} plaque-forming units per ml) (13) led to the nearly quantitative formation of a mixture of oligosaccharides (one and two repeating units) ending in reducing galactose in both cases, as determined by the method of Morrison (8). The *E. coli* K30 repeating unit tetrasaccharide (yield: 30%, wt/wt) was isolated by ion-exchange chromatography, desalted by gel filtration with a volatile buffer, and lyophilized (11); it could be sequentially degraded with β-glucuronidase from *Helix pomatia* (7) and with α-galactosidase from green coffee beans (4):



In total, these data prove that the *E. coli* K30 glycan consists of repeating units with the primary structure shown above (the arrow indicates the cleavage site of the phase-associated glycanase). In view of the generally very narrow substrate specificity of these phage enzymes (9, 11, 13), the degradation results additionally indicate the same distribution of the residual α and β linkages in the chain as in the *Klebsiella* K20 polysaccharide (3), viz., an α-mannose and a β-galactose.

The similarity or identity of the *E. coli* K30 and *Klebsiella* K20 polysaccharides is further corroborated by the finding that *E. coli* E69 and *Klebsiella* 889/50 (the serological test strain for the *Klebsiella* K20 antigen) (3, 6) strongly cross-react in slide agglutination tests with rabbit OK antisera against *E. coli* E69 and *Klebsiella* K596 (O1?:K20; the host of phage 20) (6, 13) as well as with Difco *Klebsiella* K20 serum.

This project was supported by Deutsche Forschungsgemeinschaft (H.F.) and by Fonds der Chemischen Industrie (S.S.).

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