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

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Methylation, 1H nuclear magnetic resonance, and bacteriophage degradation results indicate that the *Escherichia coli* serotype K30 capsular polysaccharide consists of \(\rightarrow\)2)-a-D-Manp-(1\(\rightarrow\)3)-\(\beta\)-D-Galp-(1\(\rightarrow\) chains carrying \(\beta\)-D-GlcUAp-(1\(\rightarrow\)3)-\(\alpha\)-D-Galp-(1\(\rightarrow\) 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

The K30 polysaccharide was extracted from *E. coli* E89 (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-gluco-uronic 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-GlcUAp-(1\(\rightarrow\)3)-D-Gal

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

<table>
<thead>
<tr>
<th>Peracetyl derivative of*</th>
<th>(T^o)</th>
<th>Primary fragments found (m/e)</th>
<th>Ratio of peak integrals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Literal</td>
<td>Found</td>
<td>45</td>
</tr>
<tr>
<td>2,4,6-ManOH*</td>
<td>2.09</td>
<td>2.15</td>
<td>+</td>
</tr>
<tr>
<td>2,4,6-GalOH</td>
<td>2.28</td>
<td>2.28</td>
<td>+</td>
</tr>
<tr>
<td>2,3,4-GlcOH</td>
<td>2.49</td>
<td>2.42</td>
<td>+</td>
</tr>
<tr>
<td>4,6-ManOH</td>
<td>3.29</td>
<td>3.38</td>
<td>+</td>
</tr>
</tbody>
</table>

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

† 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).

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

* Dideuterated fragment found instead.

split products, however, were obtained from both materials. Therefore, the *E. coli* K30 glycan was reanalyzed by methylation, gas-liquid chromatography, mass spectrometry, and 1H 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).

and that it constitutes branches at position 3 of the mannoses in a \(\rightarrow\)2)-D-Manp-(1\(\rightarrow\)3)-D-Galp-(1\(\rightarrow\) chain.

The proton magnetic resonance spectrum of the K30 glycan (1) showed, inter alia, four signals of about equal integrals at \(\delta = 4.57, 4.67, 5.20, 5.35\), indicating two \(\beta\) and two \(\alpha\) 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):

\[
\begin{align*}
\beta-D-\text{Glc}UAp \\
\alpha-D-\text{Galp} \\
\rightarrow 2)D-\text{Manp}-(1\rightarrow 3)-D-\text{Galp}-(1\rightarrow 
\end{align*}
\]

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.).

**LITERATURE CITED**


