Carbohydrate Research, 238 (1993) 335–338
Elsevier Science Publishers B.V., Amsterdam

Note

Structure of the O24 antigen of *Escherichia coli*, a neuraminic acid-containing polysaccharide

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(Received February 10th, 1992; accepted May 27th, 1992)

The O-specificity of cell wall lipopolysaccharides (LPS) is due to their polysaccharide moiety[1]. Whereas the O-specific polysaccharides are neutral in most genera, such as *Klebsiella* or *Salmonella*, they are negatively charged in a number of *Escherichia coli* strains[1,2]. Mostly, the negative charge in these O-specific polysaccharides is due to hexuronic acids or phosphate. Whereas structural analogues of *N*-acetylneuraminic acid (Neu5Ac) were reported to be the acidic constituents of LPS from *Proteus mirabilis* strains[3], Neu5Ac was known to be a constituent of capsular polysaccharides in several bacterial genera but only rarely in LPS. We have recently analysed the structures of the O104 and O56 polysaccharides of *E. coli* which both contain Neu5Ac within the polysaccharide chain[4,5]. Herein we report on the O24 polysaccharide, which also contains Neu5Ac and has a structure very similar to that of the O56 polysaccharide[5].

E. coli E41a (O24:K:H$^-$, Freiburg collection number 21735) was extracted with aqueous 45% phenol and the material obtained from the aqueous phase was subjected to ultracentrifugation[6]. The sediment contained an LPS fraction (LPS I) which consisted of short-chain LPS and R-LPS. The supernatant solution contained a LPS (LPS II) which was isolated by fractional precipitation with cetyltrimethylammonium bromide[4,5,7]. LPS II had a long O-specific polysaccharide chain, as shown by SDS-PAGE[8] (data not shown). It was used for further structural studies. GLC and the thiobarbituric acid assay[9] revealed that the O24-specific LPS II consisted of D-glucose (Glc), 2-acetamido-2-deoxy-D-glucose (GalNAc), and a thiobarbituric acid-reactive nonulosonic acid, which was identified as *N*-acetylneuraminic acid (Neu5Ac), in the molar ratios 2:1:1. For the identification of Neu5Ac, the polysaccharide was hydrolysed, and the liberated neuraminic acid was re-*N*-acetylated with acetic anhydride–sodium hydrogen car-

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TABLE I

Methylation analysis [a] of non-sialic acid sugar components of the O24 polysaccharide (PS) and the trisaccharide derived by Smith degradation (TS)

Methylation product	Sugar residue	PS	TS
1,5-Di-OAc-2,3,4,6-tetra-OMe-hexitol	Glc(1 →	1	
1,2,3,5-Tetra-OAc-4,6-di-OMe-hexitol	→ 3)Glc(1 →	1	
	2		
	↑		
1,3,5-Tri-OAc-4,6-di-OMe-2-deoxy-2-*N*-methylacetamidohexitol	→ 3)GalNAc(1 →	1	
1,3,5-Tri-OAc-2,4,6-tri-OMe-hexitol	→ 3)Glc(1 →		1
1,5-Di-OAc-3,4,6-tri-OMe-2-deoxy-2-*N*-methylacetamidohexitol	GalNAc(1 →		1

[a] Values are molar ratios.

bonate and treated with Neu5Ac aldolase–lactic acid dehydrogenase–NADH[10]. The amount of Neu5Ac so determined corresponded well with the value from the thiobarbituric acid reaction[9].

Methylation analysis of the polysaccharide[11,12] (Table I) showed that GalNAc was 3-linked, one Glc residue was terminal and nonreducing, and the other was 2,3-branched. The substitution pattern of Neu5Ac was obtained by methylation followed by formation of the methyl glycoside methyl ester derivative[13]. The mass spectrum, obtained with GLC–MS, was identical with that of *N*-acetyl-7-*O*-acetyl-*N*-methyl-4,8,9-tri-*O*-methylneuraminic acid methyl ester methyl glycoside recently obtained from the O56 polysaccharide[5]. It indicated that, as in the O56 polysaccharide, Neu5Ac was 7-linked in the O24 polysaccharide.

Periodate oxidation of the O24 polysaccharide, followed by borohydride reduction, destroyed the terminal nonreducing glucose and converted Neu5Ac into its C_8 analogue, as described in ref. 5.

Smith degradation[14] of the O24 polysaccharide resulted in a trisaccharide consisting of a terminal nonreducing GalNAc, a 3-linked glucose, and a 7-linked C_8 analogue of Neu5Ac at the reducing terminus. The structure of the last component was shown by NMR spectroscopy (see below).

The ^{13}C NMR spectrum of the O24 polysaccharide (Fig. 1) contained signals of four anomeric carbon atoms at δ 104.4, 102.7, 102.0, and 96.4. A gated decoupling experiment[15,16] showed that the signals at δ 104 and 102.7 were due to β-anomeric carbon atoms and that at δ 96.4 was due to an α-anomeric carbon atom. The signal at δ 102.0 was negative in an APT experiment[17,18], indicative of α-C-2 of Neu5Ac. In a gated decoupling experiment[15,16], the signal at δ 173.6 (C-1 of Neu5Ac) showed a coupling constant $J_{C\text{-}1,H\text{-}3ax}$ of 5.4 Hz, indicating the α-anomeric configuration of Neu5Ac[19,20]. The signals at δ 54.4 and 52.9 correspond to the nitrogen-substituted C-5 of Neu5Ac and C-2 of GalNAc, respectively, the signal at δ37.6 was due to the C-3 of Neu5Ac, and those at δ 23.3 were due to the methyl-C of the two *N*-acetyl groups. In the ^{13}C NMR spectrum of the trisaccharide, derived

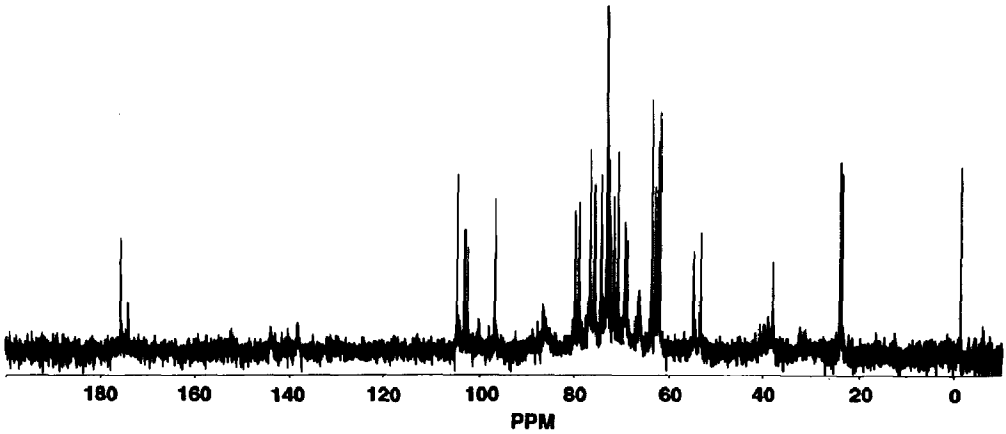

Fig. 1. 75-MHz ^{13}C NMR spectrum of the O24 polysaccharide, recorded in D_2O (70°C) with acetone (δ 31.45) as internal standard.

from the O24 polysaccharide by Smith degradation, the C-1 signal of the α-anomeric sugar residue (δ 96.4) was missing. Since the terminal nonreducing glucose was destroyed during Smith degradation, this indicated that the terminal Glc*p* was α-linked and the 2,3-disubstitued Glc*p* as well as the 3-linked GalNAc were β-linked. The signal due to C-2 of Neu5Ac, which was at δ 102.0 in the spectrum of the O24 polysaccharide, was at δ 96.8 in the spectrum of the trisaccharide. This indicated that the α-linked Neu5Ac was converted into the β anomer of its C_8 analogue at the reducing end of the trisaccharide[19].

The ^1H NMR spectrum of the O24 polysaccharide contained, inter alia, signals at δ 5.39 ($J_{H\text{-}1,H\text{-}2} < 1$ Hz), 4.67 ($J_{H\text{-}1,H\text{-}2}$ 7.5 Hz), and 4.55 ($J_{H\text{-}1,H\text{-}2}$ 8.1 Hz) corresponding to the anomeric protons of one α-linked and two β-linked sugar residues. The signal at δ 2.53 was due to the H-3*eq* of Neu5Ac. Its chemical shift value was intermediate of those for the α and β anomers (δ 2.80 and 2.40, respectively)[21], as was the distance between the signals due to H-3*ax* and H-3*eq*[22] in the α and β anomers.

The data presented here are in agreement with the following formulation of the O24 polysaccharide.

$$\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{NAc-}(1 \rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1 \rightarrow 7)\text{-}\alpha\text{-Neu5Ac-}(2 \rightarrow$$
$$\begin{array}{c} 2 \\ \uparrow \\ 1 \\ \alpha\text{-D-Glc}p \end{array}$$

Although the α-anomeric form of Neu5Ac cannot be doubted, the chemical shift values of its H-*eq* and C-3 signals are unusually high. This may be due to a close spatial apposition of the H-3 protons of Neu5Ac to the *N*-acetyl group of GalNAc. This phenomenon, which also occurred with the O56 polysaccharide[5], is under further study.

The structure of the O56-specific polysaccharide of *E. coli*[5] is remarkably similar to the structure described here. In the main chain of the O56 polysaccharide, 3-linked GlcNAc is present instead of 3-linked β-D-GalNAc and the 3-linked β-D-Glc is substituted at C-2 with α-D-Gal instead of α-D-Glc. The similarity between these two O-antigenic polysaccharides is borne out by serological cross-reactivity between *E. coli* O56 and O24. Inspection of the respective structures indicates that the cross-reacting epitope(s) must contain the common substructure → 3)-β-D-Glc*p*-(1 → 7)-α-Neu5Ac-(2 → .

ACKNOWLEDGMENTS

The technical assistance of Mrs. Sigrun Jäger is gratefully acknowledged. We thank Mrs. Helga Kochanowski for running the NMR spectra, and Mr. Dietmar Borowiak for running the mass spectra.

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