ELSEVIER

Carbohydrate Research 263 (1994) 209–215

CARBOHYDRATE RESEARCH

Structural studies of the *Escherichia coli* O90 O-antigen polysaccharide

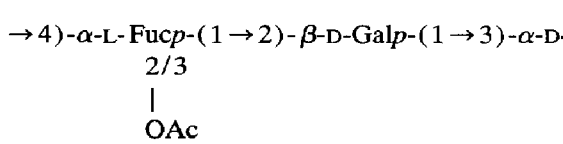
Sanjaya Ratnayake [1,a], Göran Widmalm [a,*], Andrej Weintraub [b], Ernesto C. Medina [2,b]

[a] *Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*
[b] *Karolinska Institute, Department of Immunology, Microbiology, Pathology and Infectious Diseases, Division of Clinical Bacteriology, Huddinge University Hospital, S-141 86 Huddinge, Sweden*

Received 24 January 1994; accepted 6 April 1994

Abstract

The O-specific side-chain of the lipopolysaccharide from *Escherichia coli* O90 has been investigated using methylation analysis, partial hydrolysis, and NMR spectroscopy as the principal methods. It is concluded that the polysaccharide is composed of tetrasaccharide repeating-units having the following structure.



The polysaccharide contains approximately one mole of *O*-acetyl groups per repeating unit, located on the fucose residue.

Keywords: *Escherichia coli*; Lipopolysaccharide; Blood group antigen

1. Introduction

A number of *Escherichia coli* strains possess human blood-group activity, e.g., *E. coli* O86, O90, and O127. The structure of the bacterial O-antigen of *E. coli* O86 has been

* Corresponding author.

[1] On leave from: Department of Chemistry, University of Peradeniya, Sri Lanka.

[2] On leave from: Faculty of Sciences, Universidad Nacional Autónoma de Nicaragua UNAN-León, Nicaragua, Central America.

SSDI 0008-6215(94)00152-6

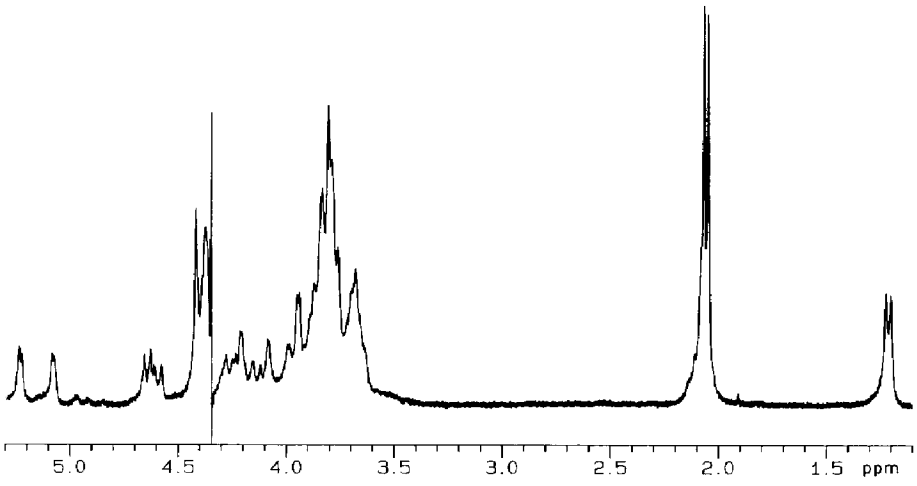


Fig. 1. The ^{1}H NMR spectrum at 270 MHz of the *O*-deacetylated *E. coli* O90 O-polysaccharide.

determined [1] and this strain has been shown to have high blood-group B activity [2]. The structure of the O-antigen from *E. coli* O127a, which shows blood-group H activity [2], was recently determined [3]. The proposed structures are in agreement with the serological typing and with immunological determinations of their blood-group activity. It has been shown that antibodies specific for the O-antigens of *E. coli* O86 and O127 also react with *E. coli* O90 [4]. This fact prompted us to investigate the structure of the O-antigenic polysaccharide from *E. coli* O90 and we here report our results.

2. Results and discussion

The lipopolysaccharide (LPS) from the *E. coli* O90 was obtained by phenol–water extraction [5]. The LPS was delipidated with acid under mild conditions to give the polysaccharide (O90 PS). An acid hydrolysate of *O*-deacetylated O90 PS, *vide infra*, contained fucose, glucose, galactose, 2-amino-2-deoxyglucose, 2-amino-2-deoxygalactose, and heptose in the relative proportions 18:13:23:5:35:6. Determination of the absolute configuration of the sugars was performed using a modification, with (+)-2-butanol, of the method developed by Leontein et al. [6] and showed L-fucose, D-galactose, and 2-amino-2-deoxy-D-galactose. The other sugars from the acid hydrolysate are attributed to derive from the core of the LPS. Methylation analysis of the O90 PS gave 2,3-di-*O*-methyl-L-fucose, 3,4,6-tri-*O*-methyl-D-galactose, and 2-deoxy-4,6-di-*O*-methyl-2-(*N*-methylacetamido)-D-galactose in the relative proportions 0.9:1.0:0.3. The ^{1}H NMR and ^{13}C NMR spectra of O90 PS showed that the O-antigen contained approximately one mole of *O*-acetyl groups distributed over at least two positions, as the methyl chemical shifts were δ_H 2.17 and 2.19 and δ_C 21.1 and 21.5. The ^{1}H NMR spectrum of the *O*-deacetylated O90 PS (Fig. 1) showed, *inter alia*, anomeric proton signals at δ 5.23, 5.08, 4.64, and 4.59 having $J_{1,2}$ values of 4.0, 3.7, 7.7, and 8.4 Hz, respectively. The ^{13}C NMR spectrum of the same material showed, *inter alia*, signals at δ 102.9 (2), 100.1, 94.1, 83.3, 77.3, 67.8, 64.5, 51.9 and 49.7.

The NMR spectra of *O*-deacetylated O90 PS indicate that it is composed of tetrasaccharide repeating-units, and the sugar and methylation analyses together with the NMR spectra show that it contains an L-fucose residue substituted through O-4, a D-galactose residue substituted through O-2, and two 2-amino-2-deoxy-D-galactose residues substituted through O-3. From methylation analysis and NMR spectral data of *O*-deacetylated O90 PS, it is deduced that all the sugars are pyranoid and that two have the α and two have the β configuration. The amino sugars are demonstrated to be *N*-acetylated by the presence of signals at δ 2.05 and 2.07 in the ^{1}H NMR spectrum and at δ 22.9, 23.3, 174.6, and 174.8 in the ^{13}C NMR spectrum. The ^{13}C chemical shifts for C-2 of the two 2-acetamido-2-deoxy-D-galactose residues indicate that one should have the β configuration, δ 51.9, and one should have the α configuration, δ 49.7. The ^{1}H,^{1}H-COSY spectrum shows that the residues with anomeric protons at δ 5.08 and 4.59 have their H-2s at δ 4.25 and 4.14, respectively, and thus belong to the α- and β-linked 2-acetamido-2-deoxy-D-galactose residues, respectively. The H-5 chemical shift of the fucose residue is δ 4.28 and hence the fucose residue is α-linked with its H-1 at δ 5.23, H-2 at δ 3.81, and H-6 at δ 1.21. The last residue with an anomeric proton signal at δ 4.62 can therefore be assigned to the β-D-galactose residue having its H-2 at δ 3.64.

The sequence of the sugars present in the polysaccharide was obtained by partial acid hydrolysis. Treatment of O90 PS with 0.1 M trifluoroacetic acid for 2 h followed by gel filtration yielded products in the oligosaccharide region. These oligosaccharides were subsequently reduced with sodium borodeuteride. One tri- and one tetra-saccharide-alditol were isolated. The trisaccharide-alditol contained D-galactose and 2-acetamido-2-deoxy-D-galactose in the relative proportions 46:54. Methylation analysis gave 2,3,4,6-tetra-*O*-methyl-D-galactose, 2-deoxy-4,6-di-*O*-methyl-2-(*N*-methylacetamido)-D-galactose and 2-deoxy-1,4,5,6-tetra-*O*-methyl-2-(*N*-methylacetamido)-D-galactitol-*1-d* in the relative proportions 71:11:18. The ^{1}H NMR spectrum showed, *inter alia*, anomeric proton signals at δ 5.13 (1 H) and 4.50 (1 H) having $J_{1,2}$ values of 4.0 and 7.3 Hz, respectively. The ^{13}C NMR spectrum of the same material showed, *inter alia*, signals at δ 105.5, 98.9, 63.5, 52.8, and 49.2. The FABMS [7] spectrum obtained in the positive mode of the underivatised trisaccharide-alditol showed a peak at m/z 612 attributed to $[M+Na]^+$. The structure of the trisaccharide-alditol is then defined by **1**.

β-D-Gal*p*-(1 → 3)-α-D-Gal*p*NAc-(1 → 3)-D-GalNAc-ol-*1-d*

1

The tetrasaccharide-alditol contained L-fucitol, D-galactose, and 2-acetamido-2-deoxy-D-galactose in the relative proportions 27:30:43. Methylation analysis gave 1,2,3,5-tetra-*O*-methyl-L-fucitol-*1-d*, 2,3,4,6-tetra-*O*-methyl-D-galactose, and 2-deoxy-4,6-di-*O*-methyl-2-(*N*-methylacetamido)-D-galactose in the relative proportions 35:50:15. The ^{1}H NMR spectrum showed, *inter alia*, signals at δ 5.09 (1 H), 4.67 (1 H), and 4.47 (1 H) having $J_{1,2}$ values of 3.7, 8.4, and 7.7 Hz, respectively. The ^{13}C NMR spectrum of the same material showed, *inter alia*, signals at δ 105.7, 103.0, 94.3, 81.7, 64.0, 51.8, and 48.8. The ^{13}C chemical shift at δ 94.3 as well as δ 64.0 should be due to a γ-gauche effect [8] between H-1 in the α-linked and H-4 in the β-linked 3-substituted 2-acetamido-2-deoxy-D-galactose residues. This effect is also observed in the *O*-deacetylated O90 PS and supports the view

that the α-linked 2-acetamido-2-deoxy-D-galactose residue substitutes the β-linked 2-acetamido-2-deoxy-D-galactose residue at O-3. The FAB mass spectrum obtained in the positive mode of the underivatised tetrasaccharide-alditol showed a peak at m/z 736 attributed to $[M+H]^+$, and fragments m/z 366 and 569 attributed to cleavage pathways abA and abcA, respectively [7]. Upon addition of sodium ions a peak is observed at m/z 758 attributed to $[M+Na]^+$. The structure of the tetrasaccharide-alditol is defined by **2**.

β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc(1→3)-β-D-Gal*p*NAc-(1→4)-L-Fucitol-*1-d*

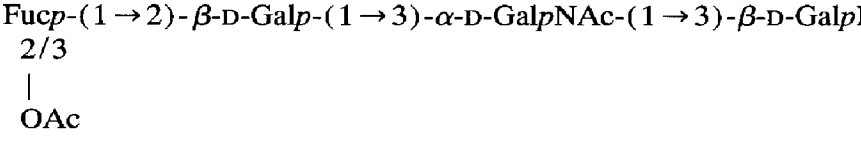
2

The above results define the repeating unit of *O*-deacetylated O90 PS as structure **3**.

→4)-α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→3)-β-D-Gal*p*NAc-(→

3

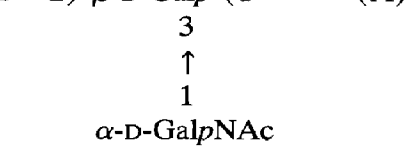
The substitution by *O*-acetyl groups at a certain sugar residue could be assessed by periodate oxidation which oxidises vicinal hydroxyl groups. Oxidation of O90 PS for 90 h at 4°C in the dark followed by addition of ethylene glycol and extensive dialysis yielded, after acid hydrolysis, fucose, galactose, and 2-acetamido-2-deoxygalactose in the ratios 1.0:0.1:1.3, i.e., the 2-substituted galactose residue was oxidised whereas the 4-substituted fucose residue was resistant to oxidation. For comparison, an acid hydrolysate of O90 PS yielded fucose, galactose, and 2-acetamido-2-deoxygalactose in the ratios 1.0:1.1:1.3, i.e., equimolar amounts of fucose and galactose. These data show that the fucose residue carries *O*-acetyl groups, which possibly were biosynthesised at one position but may have migrated during manipulations of the polymer. The repeating unit of *Escherichia coli* O90 O-antigen polysaccharide consequently has the structure **4**.



4

The determinants of the ABH blood-group system [9] are structurally defined by:

α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→ (H)



α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→ (B)
3
↑
1
α-D-Gal*p*

The structure of the O-antigen present in *E. coli* O90 shows several similarities to the structures of the O-antigens in the *E. coli* O127 and O86, **5** and **6**, respectively.

→2)-α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→3)-α-D-Gal*p*NAc-(1→
+ one mole of *O*-acetyl groups

5

→4)-α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→3)-β-D-Gal*p*NAc-(1→
3
↑
1
α-D-Gal*p*

6

The main chain of the *E. coli* O86 O-antigen is identical to the above proposed structure **3** for the *O*-deacetylated *E. coli* O90 PS. The structural determination of the *E. coli* O86 O-antigen employed degradation methods using hydrogen fluoride solvolysis followed by borodeuteride reduction or lead tetra-acetate oxidation to yield compounds having structure **1** or structure **3**, respectively. The differences in the repeating units of the O86 and O90 strains are in the type of substitutions. The *E. coli* O86 O-antigen contains a terminal nonreducing α-linked galactosyl group linked to the 3-position of the galactose in the chain. The O90 polysaccharide lacks this residue but contains acetyl group(s) linked to the fucosyl residue. The main chain of the *E. coli* O127 O-antigen is also very similar to both the O90 and O86 antigens. The main differences in the structure of the O127 antigen are: (*i*) the anomeric configuration of one of the GalNAc residues (α in O127 and β in the others) and (*ii*) the linkage of this GalNAc residue to the fucose residue, viz. 2-linked in O127 and 4-linked in the others. The reason for the cross-reactivity between these three *E. coli* strains is most likely that in all of them part of the O-antigen is very similar, e.g., α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-α-D-Gal*p*NAc-(1→3)-α/β-D-Gal*p*NAc. The two terminal sugars constitute the blood-group H antigen. Substitution of the galactosyl residue in the main chain by a terminal nonreducing galactosyl group (as in *E. coli* O86) converts the H antigen to a blood-group B antigen.

3. Experimental

General methods.—Evaporations were performed under diminshed pressure at <40°C (bath) or by flushing with air. For GLC, a Hewlett–Packard 5890A instrument, fitted with

a flame-ionisation detector, was used. Separations were performed on an HP5 fused-silica capillary column, using a temperature program 180°C (1 min) → 250°C at 3°C/min. GLC–MS was performed on a Hewlett–Packard 5890-5970 instrument, using the same phase. Hydrolysis of underivatised material was performed with 2 M trifluoroacetic acid at 120°C for 2 h. Methylation analyses were performed as previously described [10,11]. The absolute configurations of fucose, galactose, and 2-acetamido-2-deoxygalactose were determined essentially as described by Leontein et al. [5] by GLC of their glycosides, using (+)-2-butanol. A differential refractometer was used for monitoring the gel chromatography effluents. FABMS spectra in the positive mode were recorded on a Jeol SX 102 instrument, using Xe atoms (6 kV) and a matrix of glycerol, at a resolution of 1000. The polysaccharide was *O*-deacetylated using 0.1 M NaOH for 16 h at room temperature, neutralised, and purified by gel chromatography.

NMR spectroscopy.—NMR spectra of solutions in D_2O were recorded at 70°C using a Jeol GSX-270 instrument. Chemical shifts are reported in ppm relative to sodium 3-trimethylsilylpropanoate-d_4 (δ_H 0.00) and acetone (δ_C 31.00) as internal references.

Partial hydrolysis of O-deacetylated O90 PS.—The *O*-deacetylated polysaccharide (15 mg) was treated with 0.1 M trifluoroacetic acid at 100°C for 2 h. After neutralisation, the sample was freeze-dried. Gel filtration of the sample on a Bio-Gel P-2 column (2.5 × 70 cm) yielded products in the oligosaccharide region. Reduction of these oligosaccharides with sodium borodeuteride followed by conventional work-up and gel filtration yielded a tetrasaccharide-alditol (3 mg) and a trisaccharide-alditol (3 mg).

Treatment with periodate.—A solution of O90 PS (3 mg) and $NaIO_4$ (10 mg) in 0.1 M acetate buffer of pH 3.9 (10 mL) was kept in the dark for 90 h at 4°C. Ethylene glycol (0.1 mL) was then added and the sample dialysed extensively against deionised water. The sample was subjected to sugar analysis.

Bacterial strain.—*E. coli* O90:K^-:H^- (O90:CCUG 11392) was obtained from the Culture Collection, University of Göteborg, Sweden.

Isolation and purification of the O-polysaccharide.—*E. coli* O90 bacteria were grown in Ty medium (30-L culture). Bacteria were killed by the addition of formaldehyde (1% final concentration) and harvested by centrifugation. Lipopolysaccharide (LPS) was extracted by the hot phenol–water method [5]. The LPS was treated with aq 2% AcOH (pH 3.1) at 100°C for 2 h. Liberated lipid A was centrifuged, and the supernatant solution was neutralised, dialysed, and lyophilised. The product was further purified by column chromatography on a column (2.6 × 90 cm) of Bio-Gel P-6.

Acknowledgements

This work was supported by grants from the Swedish Natural Science Research Council, International Science Program in Chemical Sciences, Uppsala University, and the Swedish Agency for Research Cooperation with Developing Countries (SAREC). Dr. K. Leontein is thanked for her interest at an early stage of this project.

References

[1] M. Andersson, N. Carlin, K. Leontein, U. Lindqvist, and K. Slettengren, *Carbohydr. Res.*, 185 (1988) 211–233.
[2] G.F. Springer, P. Williamson, and W.C. Brandes, *J. Exptl. Med.*, 113 (1961) 1077–1093.
[3] G. Widmalm and K. Leontein, *Carbohydr. Res.*, 247 (1993) 255–262.
[4] H.W. Ewing, *Edwards and Ewing's Identification of Enterobacteriaceae*, 4th ed., Elsevier, New York, 1986, Chapter 6.
[5] O. Westphal and K. Jann, *Methods Carbohydr. Chem.*, 5 (1965) 83–91.
[6] K. Leontein, B. Lindberg, and J. Lönngren, *Carbohydr. Res.*, 62 (1978) 359–362.
[7] A. Dell, *Adv. Carbohydr. Chem. Biochem.*, 45 (1987) 19–72.
[8] H. Baumann, *Chem. Commun., Univ. Stockholm*, 9 (1988) 1–47.
[9] E.A. Kabat, *Am. J. Clin. Pathol.*, 78 (1982) 281–292.
[10] P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, and J. Lönngren, *Chem. Commun., Univ. Stockholm*, 8 (1976) 1–75.
[11] T.J. Waeghe, A.G. Darvill, M. McNeil, and P. Albersheim, *Carbohydr. Res.*, 123 (1983) 281–304.