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STRUCTURAL STUDIES ON THE HEXOSE REGION OF THE ENTEROBACTERIACEAE TYPE R3 CORE POLYSACCHARIDE*

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ABSTRACT

The structure of the hexose region of the Enterobacteriaceae type R3 core polysaccharide, which is the common core in *Shigella flexneri* and some *Escherichia coli* lipopolysaccharides (LPS), has been investigated. The principal method used in these studies involved methylation analysis of the original and modified materials. The polysaccharide was modified by *N*-deacetylation–deamination and by Smith degradation. As a result of these studies, the following structure is proposed:

α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→2)-[α-D-GlcNAc*p*-(1→3)]-α-D-Gal*p*-(1→3)-α-D-Glc*p*-(1→

INTRODUCTION

In the lipopolysaccharide (LPS) from Gram-negative bacteria, the lipid moiety (lipid A) is linked to the O-antigen *via* a core region. A number of different core structures have been identified in Enterobacteriaceae, and their occurrence and chemical structures have been reviewed[1]. Detailed structural information is available for some cores, but for others such information is lacking or is only tentative. One core structure, R3, seems to prevail in *Shigella flexneri*, and a partial structure (**1**) has been proposed for the hexose region of this core[2]. We now report structural studies on this core region.

α-D-Glc*p*-(1→3)-[α-D-GlcNAc*p*-(1→4)]-α-D-Gal*p*-(1→3)-[α-D-Glc*p*-(1→4)]-α-D-Glc*p*-(1→
1

RESULTS AND DISCUSSION

The LPS was isolated[3] by the method of Galanos *et al.*[4] from *Shigella flexneri* 4bR, a mutant which synthesizes a complete core but no O-antigen. The LPS was also

*Dedicated to Professor K. Heyns on the occasion of his 70th birthday.

isolated from *Escherichia coli* F653, another mutant known to synthesize a complete type R3 core. The two materials were subjected to the same structural studies. As the results were virtually identical, only those obtained with the *Sh. flexneri* material will be discussed.

The core polysaccharide (PS) was prepared from the LPS by mild acid hydrolysis. A sugar analysis of the PS showed, in agreement with previous results, that it was composed of D-glucose, D-galactose, 2-acetamido-2-deoxy-D-glucose, and heptose. The proportion between these sugars was ~3:1:1:1. As the material was not dephosphorylated, only part of the heptose is accounted for in this analysis. The hexoses, which were not isolated, were assumed to have the D configuration by analogy with numerous observations on related polysaccharides.

The ^{1}H-n.m.r. spectrum of the PS showed, *inter alia*, a signal at δ 1.97 (s), assigned to *N*-acetyl protons, and several signals in the anomeric region. Since none of these appeared at the high field with the high coupling constants expected for a β-D-linked glucopyranoside, galactopyranoside, or 2-acetamido-2-deoxy-glucopyranoside, these sugar residues are assigned α-D-linkages in the PS. This is also in agreement with the observed optical rotation, $[\alpha]_{578}^{23}$ +165° (*c* 1.0, water).

TABLE I

METHYLATION ANALYSES OF ORIGINAL AND MODIFIED POLYSACCHARIDES[a] FROM *Shigella flexneri* 4bR

O-*Methyl derivatives*	*Retention time*[b]	*Polysaccharides*[a] (*relative proportions*)		
		A	*B*	*C*
2,3,4,6-Me_4-Glc	1.00	23	30	5
2,3,4,6-Me_4-Gal	1.16			41
3,4,6-Me_3-Glc	1.72	21	25	
2,4,6-Me_3-Glc	1.76	31	25	54
3,4,6-Me_3-Gal	1.98		18	
4,6-Me_2-Gal	2.90	11		
3,4,6-Me_3-GlcNAc	4.42	15		

[a]For the original (A), *N*-deacetylated and deaminated (B), and Smith-degraded (C) polysaccharides. [b]Retention time of the corresponding alditol acetate relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol on an SP-1000 W.C.O.T. column at 220°, or on 3% OV-17 at 190° (for the GlcNAc derivative).

Methylation analysis of the PS gave the sugars listed in Table I, column A. The analysis of the 2-amino-2-deoxy-D-glucose derivative was qualitative only. Perfect stoichiometry was not expected as *N*-deacetylation, giving acid-resistant 2-amino-2-deoxyglucosidic linkages, competes with the acid hydrolysis of glycosidic linkages. The low yield of the D-galactose derivative indicates that the 2-acetamido-2-deoxy-D-glucose residue is linked to a D-galactose residue. This conclusion was confirmed

by the deamination experiments (see below). The results are in agreement with a pentasaccharide structure with two terminals, one D-glucopyranosyl and one 2-acetamido-2-deoxy-D-glucopyranosyl group, two D-glucopyranosyl residues linked through O-2 and O-3, respectively, and one D-galactopyranosyl residue, linked both through O-2 and O-3.

The PS was *N*-deacetylated and deaminated by treatment with sodium nitrite in aqueous acetic acid. Methylation analysis of this material (Table I, column B) showed that the 4,6-di-*O*-methyl-D-galactose in the analysis of the original material was replaced by 3,4,6-tri-*O*-methyl-D-galactose. The 2-acetamido-2-deoxy-D-glucopyranosyl group is consequently linked to O-3 of the branching D-galactopyranosyl residue, as in partial structure **2**.

The LPS was subjected to a Smith degradation[5], that is periodate oxidation, borohydride reduction, and acid hydrolysis under mild conditions. Sugar analysis of the product showed D-mannose, D-galactose, D-glucose, and heptose in the proportion of 23:30:33:14. The structure of the heptose region in different Enterobacteriaceae cores seems to be similar, with one terminal, one 3-linked, and one 3,7-linked L-*glycero*-D-*manno*-heptose residue[1]. The two last-named residues are phosphorylated, which explains why they are lost, partially or entirely, in methylation analysis unless the product is first dephosphorylated. The mannose in the sugar analysis just reported obviously derives from the 3-substituted heptose residue, in which the linkage between C-6 and C-7 has been cleaved by oxidation. On methylation analysis of this modified PS, extensive degradation occurred. This was avoided, however, if the product was reduced with borohydride before the methylation. Only two hexose residues in the PS, the 3-linked D-glucopyranosyl residue and the branching D-galactopyranosyl residue, should be resistant to periodate, and methylation analysis of the product (Table I, column C) demonstrates that these residues are adjacent, as in partial structure **3**.

Only two sequences of sugar residues, **4** (which also indicates the anomeric configuration of the sugar residues) and **5**, agree with the combined evidence just presented.

α-D-Glc*p*-(1→2)-α-D-Glc*p*-(1→2)-[α-D-GlcNAc*p*(1→3)]-α-D-Gal*p*-(1→3)-D-Glc*p*-(1→
4

D-Glc*p*-(1→2)-[D-GlcNAc*p*-(1→3)]-D-Gal*p*-(1→3)-D-Glc*p*-(1→2)-D-Glc*p*-(1→
5

The latter structure on Smith degradation should have yielded the disaccharide D-Gal*p*-(1→3)-D-Glc linked to O-2 of glyceraldehyde. The product, however, when

purified by gel filtration on Biogel P-2, was eluted shortly after the void volume, much earlier than di- and tri-saccharides. The presence of heptose and mannose in this product shows that partial structure **3** is linked to the heptose region, as in **4**, and not to a D-glucose residue, susceptible to periodate oxidation, as in **5**.

Our present knowledge about the Enterobacteriaceae cores[1] suggests that they are structurally related. They contain a pentasaccharide hexose region, composed of D-glucose, D-galactose, and, for some of them, 2-acetamido-2-deoxy-D-glucose, in which α-linkages predominate. In all structures which have been investigated, the innermost hexose residue, linked to a heptose, is a 3-substituted D-glucopyranose residue.

EXPERIMENTAL

General methods. — These methods were the same as in the investigation of the *E. coli* C core structure[6]. ^{1}H-N.m.r. spectra of compounds, in deuterium oxide at 85°, were recorded on a Varian XL-100 instrument.

Preparation of LPS and PS. — LPS from *Sh. flexneri* 4bR (N.C.T.C. No 8522) and *E. coli* F653 (obtained from G. Schmidt, Max-Planck-Institut für Immunbiologie, Freiburg-Zähringen, West Germany) were prepared as previously described[3,4]. LPS were treated with 1% aqueous acetic acid for 2 h at 100°, and the lipid released was removed by centrifugation. The PS were purified on a column of Sephadex G-25 with water as irrigant, and freeze-dried.

Sugar analysis. — This analysis was performed by a 3-step procedure in which the material was hydrolyzed with 2M trifluoroacetic acid for 1 h at 100°, the acid removed by distillation, and the residue acetylated. This procedure was repeated once. The sugars were analyzed as their alditol acetates[7]. The response factor was 1.0 for the neutral sugars and 0.6 for 2-acetamido-2-deoxy-D-glucose. The molar proportion of D-galactose, D-glucose, heptose, and 2-acetamido-2-deoxy-D-glucose was 14:52:18:17.

Methylation analyses. — These were performed as previously described[8]. The products were isolated by partition between chloroform and water. The identification of the different, partially methylated sugars, by g.l.c.–m.s. of their alditol acetates, was unambiguous and will not be discussed.

N-*Deacetylation–deamination*[9]. — PS (23 mg) was dissolved in dimethyl sulfoxide (5 ml) and water (1 ml). Sodium hydroxide (400 mg) and thiophenol (0.3 ml) were added and the solution was stirred for 16 h at 90°, neutralized with M hydrochloric acid, and freeze-dried. The product was purified on a Sephadex G-25 column with water as irrigant.

The *N*-deacetylated PS (10 mg) was dissolved in water (1 ml), 33% aqueous acetic acid (2 ml) and 5% aqueous sodium nitrite (2 ml) were added, and the solution was kept for 60 min at 25°, passed through a column of Dowex 50 (H^+) cation-exchange resin, and freeze-dried. Sugar analysis of this material showed D-galactose, D-glucose, and heptose in the molar proportion 18:66:16.

Smith degradation[5]. — The LPS (134 mg) was dissolved in a mixture of 0.2M sodium metaperiodate (20 ml) and 0.1M sodium acetate buffer (pH 3.9, 80 ml), and stirred for 6 days in the dark at 4°. Excess periodate was destroyed with 1,2-ethanediol (0.5 ml), and the product dialyzed for 4 h. The solution containing the modified PS was concentrated to 50 ml and sodium borohydride (500 mg) was added. After 16 h at room temperature, the pH of the solution was adjusted to ~5 by adding 4M hydrochloric acid, the solution was dialyzed for 4 h, and freeze-dried. The product was dissolved in 2M trifluoroacetic acid (20 ml) and kept for 6 h at 25°. Lipid material was removed by centrifugation and the solution was then concentrated to dryness, the product dissolved in water (2 ml) and fractionated on a column of Biogel P-2 (90 × 1.6 cm) with water as irrigant. The degraded PS (5 mg) was eluted shortly after the void volume. A hydrolyzate of this material contained D-mannose, D-galactose, D-glucose, and heptose in the molar proportion 23:30:33:14.

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REFERENCES

1 C. GALANOS, O. LÜDERITZ, E. T. RIETSCHEL, AND O. WESTPHAL, *Int. Rev. Biochem.*, 14 (1977) 239–335.
2 J. H. JOHNSTON, R. J. JOHNSTON, AND D. A. R. SIMMONS, *Biochem. J.*, 105 (1967) 79–87.
3 A. A. LINDBERG AND T. HOLME, *Acta Pathol. Microbiol. Scand.*, 80 (1972) 751–759.
4 C. GALANOS, O. LÜDERITZ, AND O. WESTPHAL, *Eur. J. Biochem.*, 9 (1969) 245–249.
5 I. J. GOLDSTEIN, G. W. HAY, B. A. LEWIS, AND F. SMITH, *Methods Carbohydr. Chem.*, 5 (1965) 361–370.
6 P. E. JANSSON, B. LINDBERG, G. BRUSE, A. A. LINDBERG, AND R. WOLLIN, *Carbohydr. Res.*, 54 (1977) 261–267.
7 J. S. SAWARDEKER, J. H. SLONEKER, AND A. R. JEANES, *Anal. Chem.*, 37 (1965) 1602–1604.
8 B. LINDBERG, *Methods Enzymol.*, 28B (1972) 178–195.
9 C. ERBING, K. GRANATH, L. KENNE, AND B. LINDBERG, *Carbohydr. Res.*, 47 (1976) C5–C7.