ACTA CHEMICA SCANDINAVICA 23 (1969) 2217–2222

Structural Studies on the O-Specific Side Chains of the Cell Wall Lipopolysaccharide from *Salmonella bredeney*

CARL GUSTAF HELLERQVIST, OLLE LARM and BENGT LINDBERG

Institutionen för organisk kemi, Stockholms Universitet, Stockholm

TORD HOLME

Bakteriologiska Institutionen, Karolinska Institutet, Stockholm

ALF A. LINDBERG

Statens bakteriologiska laboratorium, Stockholm, Sweden

The structure of the O-specific side-chains of the cell-wall lipopolysaccharide from *Salmonella bredeney* (1, 4, 12) has been investigated. Methylation analysis of the original lipopolysaccharide and of a material obtained on mild acid hydrolysis of the lipopolysaccharide have provided the essential information in this study. A detailed structure of the repeating unit of the side-chains is presented and the general structure of the side-chains in group B *Salmonella* lipopolysaccharides is discussed.

In previous communications [1,2] we have reported studies on the O-specific side-chains of cell-wall lipopolysaccharides from two strains of *S. typhimurium*, 395 MS and LT2. A structure of the repeating unit was proposed, which in some respects differed from the general structure previously suggested for group B *Salmonella* lipopolysaccharides.[3,4] The previous structure was, to a considerable extent, based upon studies on the *S. bredeney* (1, 4, 12) lipopolysaccharide.[5] For this reason and further because both *S. bredeney* and *S. typhimurium* LT 2 are amongst the most studied strains of *Salmonella* serogroup B, an investigation of the structure of the O-specific side-chains of the *S. bredeney* lipopolysaccharide has been undertaken by the same methods as used for the *S. typhimurium* lipopolysaccharides.

The lipopolysaccharide from *S. bredeney* (1, 4, 12) was isolated by phenol-water extraction and the presence of O-factors 1 and 4 in the bacteria and the lipopolysaccharide was established as previously described.[1] The presence of O-factor 12_2 could not be demonstrated. The methods used in the chemical studies were the same as employed in the earlier investigation on the *S. typhimurium* 395 MS lipopolysaccharide,[1] and will only be briefly described.

The polysaccharide was subjected to a two-step hydrolysis, and the sugars in the hydrolysate analysed by GLC [6] as their alditol acetates. The relative proportions of D-glucose, D-galactose, D-mannose, L-rhamnose, and abequose were 10:25:23:22:20. The sugars have been fully identified by Krüger *et al.*[7] and, in the present study, their identity was established by mass spectrometry of the alditol acetates.[8] The lipopolysaccharide did not contain *O*-acetyl groups, in agreement with the absence of O-factor 5.

The fully methylated lipopolysaccharide, prepared by methylation with methylsulphinyl carbanion-methyl iodide in methyl sulphoxide,[9] was hydrolysed, the methylated sugars converted into their alditol acetates and analysed by GLC [10]—mass spectrometry.[11] The results are given in Table 1 and a typical chromatogram, on an ECNSS-M column, is given in Fig. 1. Mass

Table 1. Methyl ethers from the hydrolysate of methylated lipopolysaccharide.

Sugars	Peak	T [a]	mole % [b]
2,4-Di-*O*-methyl-abequose	A	0.32	11.9
2,3-Di-*O*-methyl-L-rhamnose	B	0.99	22.0
2,3,4,6-Tetra-*O*-methyl-D-glucose	B	1.00	5.0
2,3,4,6-Tetra-*O*-methyl-D-galactose	C	1.22	3.0
2,4,6-Tri-*O*-methyl-D-mannose	D	2.05	1.5
2,4,6-Tri-*O*-methyl-D-galactose	E	2.27	15.0
3,4,6-Tri-*O*-methyl-D-galactose	F	2.50	1.1
4,6-Di-*O*-methyl-D-mannose	G	3.30	23.1
3,6-Di-*O*-methyl-D-galactose	H	4.30	1.5
2,4-Di-*O*-methyl-D-glucose	I	5.10	2.7
2,4-Di-*O*-methyl-D-galactose	K	6.35	4.6

[a] Retention times of the corresponding alditol acetates on the ECNSS—M column relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol.

[b] As a considerable amount of 2,4-di-*O*-methyl-abequose was lost during the methylation analysis, the mole % of the methylated sugars are given relative to that of 2,3-di-*O*-methyl-L-rhamnose, which it is assumed represents the mole % of L-rhamnose in the original lipopolysaccharide.

spectra were taken at the beginning, maximum, and end of each main peak, and at the maxima of the minor peaks.

The two components in peak B, which were not resolved on the ECNSS-M column, were well separated on an OS 138 column. With one exception, the methylated sugars identified were the same as those found in the methylated *S. typhimurium* lipopolysaccharide.[1,2].

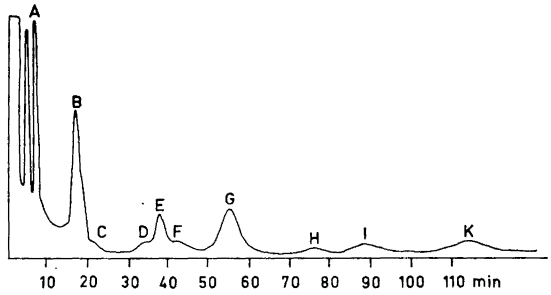

Fig. 1. GLC separation on an ECNSS-M column at 170° of the methylated sugars, as their alditol acetates, obtained from the hydrolysate of the fully methylated *S. bredeney* lipopolysaccharide.

2,4-Di-*O*-methyl-D-galactose, which was not found in the methylated *S. typhimurium* lipopolysaccharide, was identified from the mass spectrum and *T*-value (6.35) of its alditol acetate. The *T*-values for the corresponding D-mannose and D-glucose derivatives are 5.42 and 5.10, respectively. The percentages of 2,4-di-*O*-methyl-D-galactose and 2,3,4,6-tetra-*O*-methyl-D-glucose are approximately equal which substantiates the assertion that the presence of O-factor 1 is associated with a terminal α-D-glucopyranose residue in the 6-position of D-galactose. The *S. enteritidis* lipopolysaccharide,[12] also containing O-factor 1, on methylation analysis yielded the same di-*O*-methyl-D-galactose. The fact that 2,6-di-*O*-methyl-D-galactose was not obtained is consistent with the virtual absence of O-factor 12_2.

One of the methyl ethers which had previously been described as a 3,4,6-tri-*O*-methyl-hexose, has now been identified as the D-galactose derivative, as the *T*-value of its alditol acetate (2.50) differs from those of the corresponding D-glucose (2.11) and D-mannose (1.95) derivatives.

The ratio of the molar percentages of D-glucose, D-galactose, D-mannose, L-rhamnose, and abequose, calculated from the methylation analysis, and assuming that the molar percentage of L-rhamnose is the same as in the original polysaccharide, is 8:26:25:22:12. The agreement between these values and those obtained from the sugar analysis is, except for abequose, good. It seems probable that a considerable amount of the volatile 2,4-di-*O*-methyl-abequose and derivatives was lost while concentrating the solutions.

A sample of the lipopolysaccharide was subjected to a mild acid treatment in order to hydrolyse all the abequosidic linkages and part of the L-rhamnosidic linkages, leaving most of the other linkages intact. A methylation analysis was performed on the resulting polymeric and oligomeric material (Table 2). As with the *S. typhimurium* lipopolysaccharide, it was found that virtually all the 4,6-di-*O*-methyl-D-mannose had disappeared and been replaced by 3,4,6-tri-*O*-methyl-D-mannose, and that part of the 2,4,6-tri-*O*-methyl-D-galactose had been replaced by 2,3,4,6-tetra-*O*-methyl-D-galactose. The results show that, in the original lipopolysaccharide, abequose is linked to D-mannose in the 3-position and L-rhamnose to D-galactose in the 3-position.

Table 2. Methyl ethers from the hydrolysate of methylated, degraded lipopolysaccharide.

Sugars	T [a]	mole % [b]
2,3-Di-*O*-methyl-L-rhamnose	0.99	22.0
2,3,4,6-Tetra-*O*-methyl-D-glucose	1.00	6.1
2,3,4,6-Tetra-*O*-methyl-D-galactose	1.22	8.0
3,4,6-Tri-*O*-methyl-D-mannose	1.95	23.5
2,4,6-Tri-*O*-methyl-D-galactose	2.27	8.6
2,3,4-Tri-*O*-methyl-D-galactose	3.45	2.4
2,4-Di-*O*-methyl-D-glucose	5.10	2.4
2,4-Di-*O*-methyl-D-galactose	6.35	2.0

[a] See Table 1.
[b] The mole % of the methylated sugars are given relative to that of 2,3-di-*O*-methyl-L-rhamnose, which it is assumed represents the mole % of L-rhamnose in the original lipopolysaccharide.

The small amount of 2,4,6-tri-*O*-methyl-D-mannose in the hydrolysate of the methylated original lipopolysaccharide is almost certainly derived from the 3-*O*-abequosyl-D-mannose residues which terminate the O-specific side-chains. The percentage of this sugar (1.5) and of 4,6-di-*O*-methyl-D-mannose (23.1) leads to an average number of repeating units in the side-chains of approximately 16. Some of the other minor components are most probably derived from the basal core of the lipopolysaccharide.

A sample of the lipopolysaccharide, in 0.05 M aqueous sulphuric acid, was kept at 80° and the change in optical rotation was followed. A rapid decrease from $[\alpha]_{578}$ +70° to +65° over 2 h, followed by a slower decrease to +48° over 22 h, was observed. Because of the low solubility of the lipopolysaccharide these figures are not very accurate but they show that the abequose residues, being the most readily hydrolysed, are α-linked and that the L-rhamnosidic linkages, being the next most readily hydrolysed, are β-linked. Analogous results were obtained from the *S. typhimurium* lipopolysaccharides.[1,2]

From the present results, a detailed structure of the O-specific side-chains in the *S. bredeney* lipopolysaccharide is proposed (Fig. 2). The anomeric nature of the D-glucose, D-galactose, and D-mannose residues are assumed to be the same as in the closely related lipopolysaccharide studied by Staub *et al.*[3-5] The other structural features, including the fact that the terminal

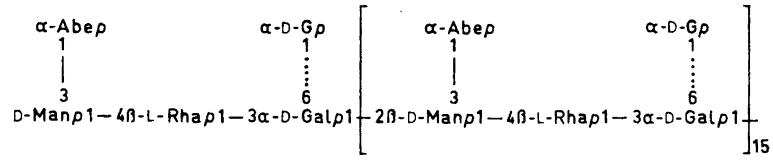

Fig. 2. Proposed structure for the O-specific side-chains of *Salmonella bredeney.*

residue on the biological repeating unit consists of a 3-*O*-abequosyl-D-mannose residue, have been established in the present study.

These results, in conjunction with those from similar studies on the *S. typhimurium* lipopolysaccharides strongly suggest that the tetrasaccharide repeating unit, depicted in Fig. 3, is typical for group B *Salmonella* lipopolysac-

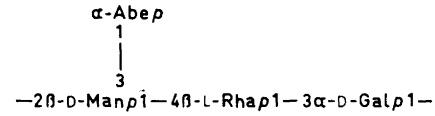

Fig. 3. Structure of the tetrasaccharide residue, common to the O-specific side-chains of group B *Salmonella* lipopolysaccharides.

charides in general. The presence of O-factor 4 in this group is associated with the α-abequose residue linked to the 3-position of D-mannose. The D-galactose residues may be substituted with an α-D-glucopyranose residue in the 6-position (O-factor 1) or in the 4-position (O-factor 12_2). All, or part, of the abequose residues may be acetylated in the 2-position (O-factor 5).

EXPERIMENTAL

The experimental methods used in this investigation were the same as used in the investigation of the *S. typhimurium* 395 MS.[1] The *Salmonella bredeney* (1, 4, 12) strain, the same as used by Staub *et al.*, was obtained from Dr. L. Le Minor, Institut Pasteur, Paris. The cultivation yield of the *S. bredeney* strain was 15.0 g, (4 l culture volume). The yield of cell walls was 2.95 g and of lipopolysaccharide 470 mg (dry weights). The lipopolysaccharide preparation was checked by the passive hemagglutination inhibition test using anti O:1, anti O:4, and anti O:12_2 serum. The minimal concentrations needed for inhibition (μg/ml) were: less than 1 μg for the *S. bredeney* lipopolysaccharide/*S. paratyphi* A O I.S. 256 anti O:1 factor serum system; less than 1 μg for the *S. typhimurium* 395 MS lipopolysaccharide/*S. san-diego* I.S. 111 anti O:4 factor system and more than 256 μg for both the *S. typhimurium* 395 MS lipopolysaccharide/*S. paratyphi* B O I.S. 248 anti O:5 factor system and the *S. typhimurium* 395 MS lipopolysaccharide/*S. typhi* T4 I.S.58 anti O:12_2 factor system.

Acknowledgements. We are indebted to Professor Gunnar Widmark, Department of Analytical Chemistry, Stockholm University, for the mass spectrometry facilities placed at our disposal. The skilled technical assistance of Miss Mary Andersson, Miss Gunnel Ljunggren and Mrs. Kerstin Norrgren is also gratefully acknowledged. This work was supported by grants from the *Swedish Medical Research Council* (to T.H. and A.A.L. No. B 68—48 x—656—03 to C.G.H., O.L. and B.L. No. B 69—13 x—2522—01), from the *Swedish Natural Science Research Council*, from *Harald Jeanssons stiftelse*, and from *Emil and Vera Cornells stiftelse.*

REFERENCES

1. Hellerqvist, C. G., Lindberg, B., Svensson, S., Holme, T. and Lindberg, A. A. *Carbohyd. Res.* **8** (1968) 41.
2. Hellerqvist, C. G., Lindberg, B., Svensson, S., Holme, T. and Lindberg, A. A. *Carbohyd. Res.* **9** (1969) 237.

3. Staub, A. M. and Bagdian, G. *Ann. Inst. Pasteur* **110** (1966) 849.
4. Le Minor, L. and Staub, A. M. *Ann. Inst. Pasteur* **110** (1966) 834.
5. Bagdian, G., Lüderitz, O. and Staub, A. M. *Ann. N.Y. Acad. Sci.* **133** (1966) 405.
6. Sawardeker, J. S., Sloneker, J. H. and Jeanes, A. R. *Anal. Chem.* **12** (1965) 1602.
7. Krüger, L., Lüderitz, O., Strominger, J. L. and Westphal, O. *Biochem. Z.* **335** (1962) 548.
8. Chizhow, O. S., Golovkina, L. S. and Wulfson, N. S. *Izv. Akad. Nauk. SSSR Ser. Khim.* **1966** 1915.
9. Hakomori, S. *J. Biochem. (Tokyo)* **55** (1964) 205.
10. Björndal, H., Lindberg, B. and Svensson, S. *Acta Chem. Scand.* **21** (1967) 1801.
11. Björndal, H., Lindberg, B. and Svensson, S. *Carbohyd. Res.* **5** (1967) 477.
12. Hellerqvist, C. G., Lindberg, B., Svensson, S., Holme, T. and Lindberg, A. A. *Acta Chem. Scand.* **23** (1969) 1588.

Received December 20, 1968.