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Preliminary communication

More on the structure of *Vibrio cholerae* O22 lipopolysaccharide

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Abstract

The structure of a short-chain lipopolysaccharide (LPS) of *Vibrio cholerae* O22 strain 169-68, that cross-reacts with *V. cholerae* O139 Bengal, was elucidated. The structure differs in detail from that reported on another strain of O22 [A.D. Cox, J-R. Brisson, P. Thibault and M.B. Perry, *Carbohydr. Res.*, 304 (1997) 191–208]. The similarity and difference between the LPS structures of the two strains as well as between O22 and O139 are discussed.

Keywords: *Vibrio cholerae*; Lipopolysaccharide; O-antigen

Vibrio cholerae O22 is one of the bacteria which are serologically related to *V. cholerae* O139 Bengal [1], the second (after O1) *V. cholerae* serotype that causes cholera. Elucidation of the structure of the lipopolysaccharide (LPS) of *V. cholerae* O22 strain NRCC 4904 [2] demonstrated that its O-chain has partial structures in common with *V. cholerae* O139 LPS [3,4], which may be responsible for the serological cross-reactivity. Our studies on the LPS of another strain of *V. cholerae* O22 (169-68, a gift from Professor T. Holme, Karolinska Institute, Stockholm) further confirmed the structural similarity between LPSs of the two serotypes and added some more details about the *V. cholerae* O22 LPS structure.

Unlike in strain NRCC 4904, no LPS species lacking the O-chain was found in strain 169-68. Chemical analysis of the strain 169-68 LPS revealed the same components as in *V. cholerae* O139 LPS [4], including 2-aminoethyl phosphate (*P*Etn) but excluding galactose-4,6-cyclophosphate (Gal4,6*P*). Strong alkaline degradation of the *O*-deacylated LPS (4 M KOH, 100 °C, 8 h) was accompanied by the loss of a part of the O-chain owing to β-elimination in the 4-substituted GalA residue and gave a mixture of tridecasaccharide tris- and tetrakis-phosphates. As judged by 1D ^{1}H and ^{13}C NMR and ESI MS data, these were essentially the same as those obtained from *V. cholerae* O139 LPS [4]. Thus, signals were observed inter alia for C-6 of QuiN (δ 17.6), C-3 of Kdo (δ 35.6), C linked to N (δ 55.3–55.6, 4 C), C-1 of GlcN1*P* (δ

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92.1), C-4 and C-5 of hex-4-enuronic acid (δ 106.9 and 147.3, respectively). The only difference was that the content of the tetrakisphosphate with a 3-deoxy-D-*manno*-octulosonic acid (Kdo) bisphosphate derived from Kdo4*P*7*P*Etn [4] was markedly lower and estimated at about 20%, fitting with the content of 2-aminoethanol in the initial LPS.

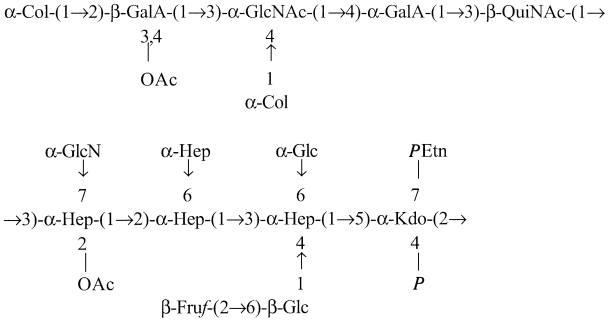
Delipidation of the strain 169-68 LPS with 0.1 M sodium acetate buffer at pH 4.2 required 4 h at 100 °C and resulted in almost complete loss of fructose (Fru) and one colitose residue (Col) linked to GlcNAc (OS1), while after 1 h at 105 °C both Col residues were affected only insignificantly and more than half of Fru remained (OS2). Methylation analysis [5], ESI MS, ^{1}H and ^{13}C NMR spectroscopic studies showed that OS1 had basically the same structure as that proposed for the delipidated LPS sample of strain NRCC 4904 [2]. Most importantly, we could confirm the presence of two GalA, two Col, one GlcNAc and one QuiNAc residue in the O-chain and in total two *O*-acetyl groups. Characteristic ^{13}C NMR chemical shifts for some signals were observed at δ 16.6 (Col C-6), 17.7 (QuiNAc C-6), 21.4 (OAc), 23.2–23.4 (NAc), 34.0 (Col C-3), 41.2 (*P*Etn), 54.6 (QuiNAc C-2), and 55.5 (GlcNAc C-2).

The analyses revealed heterogeneity in both OS1 and OS2, which was associated with partial substitution with terminal L-*glycero*-D-*manno*-heptose (Hep) residues estimated as about 75%. This followed from identification of methylated Hep derivatives derived from the 2,6-disubstituted Hep and 2-substituted Hep units in the molar ratio ~3:1, respectively.

Accordingly, together with the ions of the major species in OS2, $(Col)_2(GalA)_2(GlcNAc)(QuiNAc)(Hep)_4(Glc)_2(Fru)(GlcN)(anhydro\text{-}Kdo)(Ac)_2$, and of that lacking Fru with the molecular masses 2723 and 2561 Da, respectively, the ESI mass spectrum showed ions of two corresponding minor species having molecular masses by 192 Da less and, hence, containing only three Hep residues. Substitution with terminal GlcN was complete, at variance with what was claimed for the LPS from strain NRCC 4904 [2]. Also, our studies could not confirm any third Glc residue which was suggested to be present in LPS of strain NRCC 4904; a minor product claimed to contain three hexose residues which was detected by ESI MS in the delipidated sample from strain NRCC 4904 [2], may well contain two Glc and one Fru residue coming from incomplete removal of Fru during degradation of LPS at pH 4.2.

Methylation with methyl iodide in dimethyl sulfoxide using a short treatment time (5 min) of the sample with methylsulfinylmethanide or methylation with methyl trifluoromethanesulfonate in trimethyl phosphate in the presence of 2,6-di(*tert*-butyl)pyridine [6] revealed 2,3,7-trisubstituted Hep instead of 3,7-disubstituted Hep which appeared when the conventional methylation protocol [5] was applied; thus, *O*-acetylation at position 2 was demonstrated.[1]

Therefore, the following structure is proposed for the *V. cholerae* O22 strain 169-68 LPS O-chain and core linked via the glycosidic linkage of 2-acetamido-2,6-dideoxy-D-glucose (QuiNAc):



where substitution with terminal Hep and *P*Etn is nonstoichiometric; the monosaccharide residues have D configuration and, unless are otherwise stated, present in the pyranose form. The position of the *O*-acetyl group at β-GalA and the β-configuration of Fru were not confirmed and shown in the formula according to published data for *V. cholerae* O22 strain NRCC 4904 [2] and *V. cholerae* O1 [7], respectively.

Thus, there is no significant difference in the core structures of *V. cholerae* O22 and O139 LPSs; their O-chains are similar and differ only in the presence of an *O*-acetylated β-GalA in the former instead of β-Gal4,6*P* in the latter and in the anomeric configuration of GlcNAc, as already shown by Cox et al. [2]. Additional immunochemical studies are necessary to reveal the exact epitope specificity of these LPS antigens.

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[1] The same location, unknown previously, was determined by us for the *O*-acetyl group in the core of *V. cholerae* O139 LPS.

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References

[1] T. Shimada, E. Arakawa, K. Itoh, T. Nakazato, T. Okitsu, S. Yamai, M. Kusum, G.B. Nair, and Y. Takeda, *Curr. Microbiol.*, 29 (1994) 331–333.
[2] A.D. Cox, J.-R. Brisson, P. Thibault, and M.B. Perry, *Carbohydr. Res.*, 304 (1997) 191–208.
[3] A.D. Cox and M.B. Perry, *Carbohydr. Res.*, 290 (1996) 59–65.
[4] Y.A. Knirel, G. Widmalm, S.N. Senchenkova, P.-E. Jansson, and A. Weintraub, *Eur. J. Biochem.*, 247 (1997) 402–410.
[5] A.B. Blakeney and B.A. Stone, *Carbohydr. Res.*, 140 (1985) 319–324.
[6] P. Prehm, *Carbohydr. Res.*, 78 (1980) 372–374.
[7] E.V. Vinogradov, K. Bock, O. Holst, and H. Brade, *Eur. J. Biochem.*, 233 (1995) 152–158.