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Note

Structural studies of the O-antigenic oligosaccharide from *Vibrio salmonicida* strain C2 isolated from Atlantic cod, *Gadus morhua* L.

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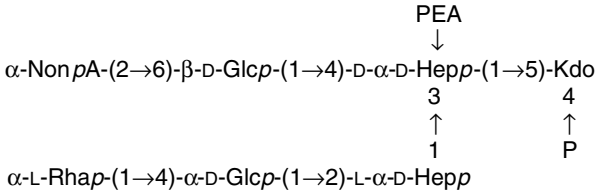
Abstract—We report the chemical structure of the oligosaccharide part of *Vibrio salmonicida* lipopolysaccharide serotype C2, isolated from Atlantic cod (*Gadus morhua* L.). The structure was established by NMR spectroscopy and mass spectrometry. It is concluded that the oligosaccharide has the following structure in which L-α-D-Hep*p* is L-*glycero*-α-D-*manno*-heptopyranose, D-α-D-Hep*p* is D-*glycero*-α-D-*manno*-heptopyranose, α-NonA is 5-acetamidino-7-acetamido-3,5,7,9-tetradeoxy-L-*glycero*-α-D-*galacto*-nonulosonic acid, and PEA is phosphoethanolamine.



Keywords: *Vibrio salmonicida*; Coldwater vibriosis; Lipopolysaccharide (LPS); Cod; *Gadus morhua* L.

Coldwater vibriosis or 'Hitra disease' was first reported in farmed Atlantic salmon in the late 1970s in northern Norway where outbreaks resulted in substantial losses of fish in seawater farms.[1] The disease may be classified as an acute hemorrhagic septicaemia. The causative agent is a psychrophilic Gram-negative bacterium called *Vibrio salmonicida*.[2] Isolates of *V. salmonicida* from Atlantic salmon comprise a biochemically and serologically homogenous group of motile pleomorphic curved rods that are oxidase and catalase positive.[2,3] *V. salmonicida* is also recognised in aquacultured Atlantic salmon in Scotland, in Eastern Canada, and in the Shetland and Faroe Islands.[4] Affected fish stop feeding, and they are lethargic and dark coloured. They show extensive petechial hemorrhages with oedema in the vent region. The visceral organs show extensive hemorrhaging including the liver, kidney, caeca, and abdomial fat. No virulence mechanisms are at present documented for *V. salmonicida*.[5–7] In contrast to another fish pathogen, *Vibrio anguillarum*, causing 'classical' vibriosis, no extracellular products like hemolysins and proteolytic enzymes have been found in culture filtrates of *V. salmonicida*. Light and electron microscopic studies showed necrotic tissues in the kidneys and bacteria in contact with injured endothelial cells.[8,9] Vaccines developed against coldwater vibriosis have been shown to give full protection against infection with *V. salmonicida*.[10] In spite of this, outbreaks of coldwater vibriosis occasionally occur in vaccinated Atlantic salmon.[10] Although predominantly a disease of Atlantic salmon, outbreaks have also been reported in

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sea-cultured rainbow trout (*Oncorhynchus mykiss*) and farmed cod.[11] By using a panel of monoclonal antibodies, *V. salmonicida* isolated from cod could be classified into separate serogroups, C1 and C2, whereas all the isolates from Atlantic salmon were of the same serotype and identical to C1.[12–14]

Hydrolysis of the OS with 2 M trifluoroacetic acid for 1 h at 120 °C yielded L-rhamnose, D-glucose, and L-*glycero*-D-*manno*-heptose in approximate proportions 1:2:1 as indicated by GLC of the alditol acetates. The structure of the oligosaccharide from *V. salmonicida* strain C2, an isolate from cod, bears close resemblance to that of C1, an isolate from Atlantic salmon.[15] Our previous studies showed that the C1 oligosaccharide contains a 4,6-dideoxy-4-[(*R*)-3-hydroxybutaneamido]-D-galactose (Fuc*p*4NBA).[15] This part is absent in C2, the other parts of the structure are the same. The NMR ^{13}C and ^{1}H spectra of C1 and C2 oligosaccharides are quite similar except that all the signals from Fuc*p*4NBA are lacking in C2 [^{1}H NMR (Fig. 1) and ^{13}C NMR (Fig. 2) spectra of the oligosaccharide from C2]. A FAB mass spectrum of the OS showed a pseudomolecular ion, $[M+H]^{+}$, at *m*/*z* 1511.6 (Fig. 3). The molecular mass of an oligosaccharide comprising two hexoses, one deoxyhexose, two heptoses, one anhydro-KDO, one nonulosonic acid residue with one acetamido, and one acetamidino and one PEA group is 1512. The mass spectra show that the 4,6-dideoxy-4-hydroxybutaneamidohexose present in C1 is absent in C2 (Fig. 4).

V. salmonicida isolated from Atlantic salmon has a rough type of LPS as analysed by SDS-polyacrylamide gel electrophoresis, and the oligosaccharide moiety has a molecular mass of 1867.8.[13,15] Eleven of fifteen monoclonal antibodies made against *V. salmonicida* reacted with LPS, and the remaining four reacted with a protein antigen of 24 kD showing that LPS is a dominant anti-

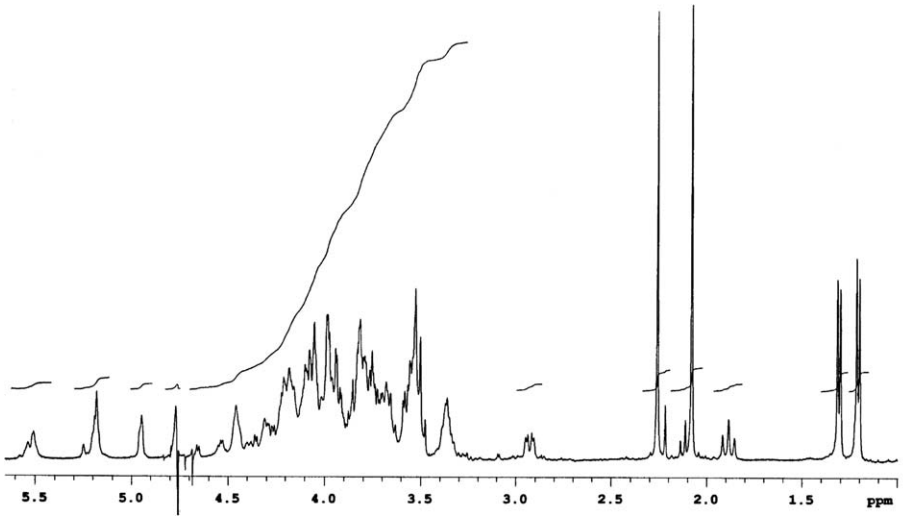

Figure 1. The ^{1}H NMR spectrum of the *V. salmonicida* strain C2 OS.

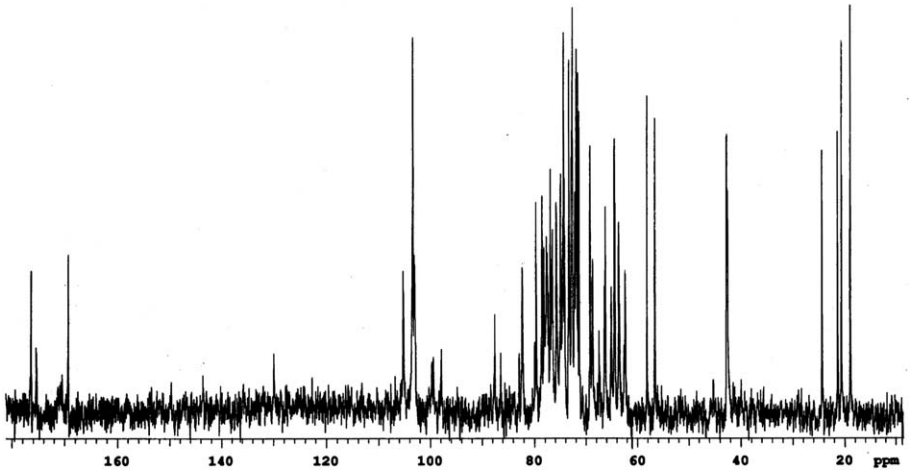

Figure 2. The ^{13}C NMR spectrum of the *V. salmonicida* strain C2 OS.

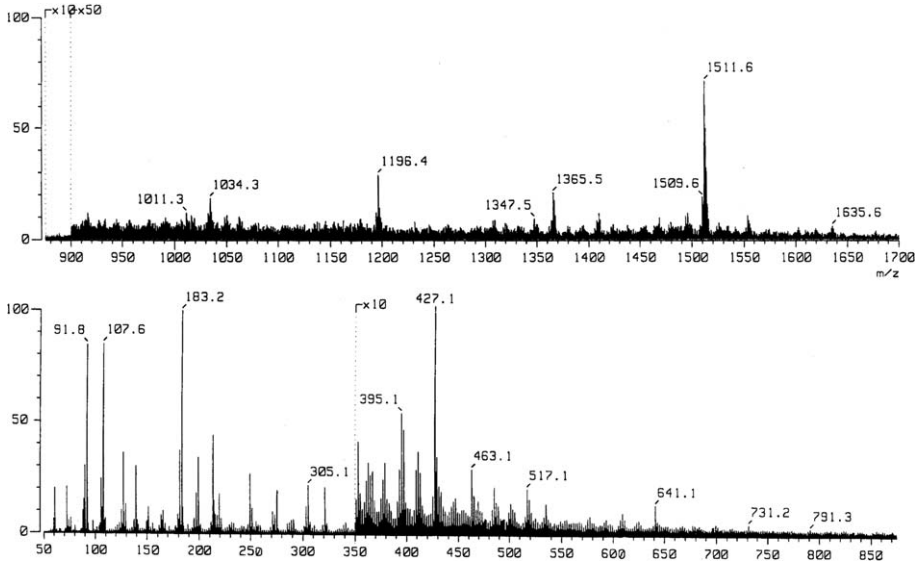

Figure 3. The FABMS spectrum of the *V. salmonicida* strain C2 OS.

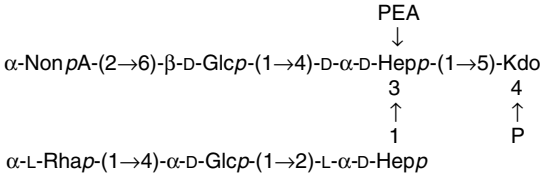

Figure 4. Structure of the lipopolysaccharide of *V. salmonicida* serotype C2 isolated from cod. L-α-D-Hep*p* is L-*glycero*-α-D-*manno*-heptopyranose, D-α-D-Hep*p* is D-*glycero*-α-D-*manno*-heptopyranose, α-NonA is 5-acetamidino-7-acetamido-3,5,7,9-tetradeoxy-L-*glycero*-α-D-*galacto*-nonulosonic acid, Glc*p* is glucopyranose, Rha*p* is rhamnopyranose, Kdo is 3-deoxy-D-*manno*-octulosonic acid and PEA is phosphoethanolamine.

gen of the bacterium.[13] Thirty isolates of *V. salmonicida* from Atlantic salmon and nine isolates from Atlantic cod were serotyped with a panel of 13 monoclonal antibodies shown to react with LPS determinants.[12,13] All isolates from Atlantic salmon expressed epitopes that reacted with the monoclonals (serotype C1).[14] In contrast, only six of the nine isolates from Atlantic cod reacted identically to serotype C1. The remaining three isolates reacted only to four of the thirteen monoclonals (serotype C2).[14] In the present study, we have shown that α-D-Fuc*p*4NBA is missing in LPS from C2, and the absence of this monosaccharide dramatically alters the antigenicity of this serotype compared with serotype C1.[14]

1. Experimental

V. salmonicida serotype C2 (LFI 1237)[14] was grown at 12 °C for 48 h on an orbital shaker in fluid medium containing marine broth (37.4 g L^{-1} (Difco, USA) supplemented with 5 g L^{-1} proteose peptone (Marine Biochemicals, Tromsø, Norway) and 20 g L^{-1} NaCl. The growth conditions were exactly the same as for growth of strain NCMB 2262 used in our previous studies on the structure of LPS.[15] LPS was prepared by the phenol–chloroform–light petroleum procedure of Galanos et al.[16] The yield of LPS was 1.5 % (w/w) of the dried bacteria. Treatment of the LPS in a 0.1 M acetate buffer of pH 4.4 containing 0.1% sodium dodecyl sulfate (SDS) for 2 h at 100 °C released the oligosaccharide (OS), which was purified by gel-permeation chromatography. The NMR techniques and mass spectrometric methods are described.[15]

References

1. Egidius, E.; Andersen, K.; Clausen, E.; Raa, J. *J. Fish Dis.* **1981**, *4*, 353–354.
2. Egidius, E.; Wiik, R.; Andersen, K.; Hoff, K. A.; Hjeltnes, B. *Int. J. Syst. Bacteriol.* **1986**, *36*, 518–520.
3. Holm, K. O.; Strøm, E.; Stensvåg, K.; Raa, J.; Jørgensen, T. *Fish Pathol.* **1985**, *20*, 125–129.
4. Bruno, D. W.; Hastings, T. S.; Ellis, A. E. *Dis. Aquat. Org.* **1986**, *1*, 163–168.
5. Colquhoun, D.; Sørum, H. *Fish Shellfish Immunol.* **1998**, *8*, 367–377.
6. Sørum, H.; Poppe, T. T.; Olsvik, Ø. *J. Clin. Microbiol.* **1988**, *26*, 1679–1683.
7. Wiik, R.; Andersen, K.; Daae, F. L.; Hoff, K. A. *Appl. Environ. Microbiol.* **1989**, *55*, 819–825.
8. Soleim, Ø. *Bull. Eur. Assoc. Fish. Pathol.* **1985**, *5*, 88–90.
9. Totland, G. K.; Nylund, A.; Holm, K. O. *J. Fish Dis.* **1988**, *11*, 1–13.
10. Eggset, G.; Mikkelsen, H.; Killie, J. E. A. *Fish Shellfish Immunol.* **1997**, *7*, 247–260.
11. Jørgensen, T.; Midling, K.; Espelid, S.; Nilsen, R.; Stensvåg, K. *Bull. Eur. Assoc. Fish Pathol.* **1989**, *9*, 42–44.
12. Espelid, S.; Holm, K. O.; Hjelmeland, K.; Jørgensen, T. *J. Fish Dis.* **1988**, *11*, 207–214.

13. Bøgwald, J.; Stensvåg, K.; Hoffman, J.; Espelid, S.; Holm, K. O.; Jørgensen, T. *J. Fish Dis.* **1990**, *13*, 293–301.
14. Schrøder, M. B.; Espelid, S.; Jørgensen, T. *Fish Shellfish Immunol.* **1992**, *2*, 211–221.
15. Edebrink, P.; Jansson, P.-E.; Bøgwald, J.; Hoffman, J. *Carbohydr. Res.* **1996**, *287*, 225–245.
16. Galanos, C.; Lüderitz, O.; Westphal, O. *Eur. J. Biochem.* **1969**, *9*, 245–249.