Structural studies of the O-antigen polysaccharide from *Escherichia coli* O152

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Introduction

The structure of the repeating unit of the O-antigen polysaccharide (PS) from *Escherichia coli* O152 has been solved, using component analysis and nuclear magnetic resonance (NMR) spectroscopy.

E. coli O152 is an enteroinvasive *E. coli* bacterium (EIEC) [1] that causes severe diarrhea [2,3]. In order to make conclusions about resemblance to other bacteria it is important to perform serology and cross-reactivity studies. Consequently, knowledge about the structure of the bacterial O-antigen is essential.

Results

Component analysis and NMR (figure 1) showed that the PS is built up of five different sugar residues, α-D-Glc*p*NAc (**A**), α-D-Glc*p* (**B**), β-L-Rha*p* (**C**), β-D-Glc*p*NAc (**D**) and β-D-Glc*p* (**E**).

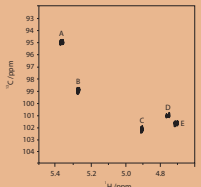

Figure 1. Part of the ^{1}H,^{13}C-HSQC spectrum showing the anomeric region.

The anomeric proton from residue **A** has an extra splitting (7 Hz) in the ^{1}H-NMR spectrum, indicating the presence of an phosphodiester, which was confirmed by 1D ^{31}P and ^{1}H,^{31}P-COSY experiments (figure 2).

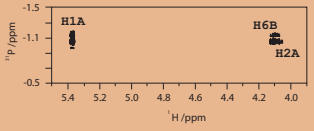

Figure 2. The cross-peaks in the ^{1}H,^{31}P-COSY spectrum.

Residue **C** is concluded to be terminal because the chemical shift do not diverge significantly from unsubstituted β-L-Rha*p*.

The chemical shifts were assigned using a combination of 1D and 2D homonuclear and heteronuclear NMR experiments. The linkage pattern was determined by ^{1}H,^{13}C-HMBC and confirmed by ^{1}H,^{1}H-NOESY experiments. The proposed structure of the repeating unit is depicted in figure 3 (below).

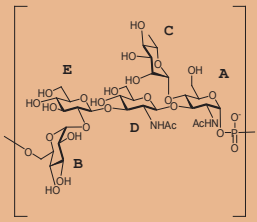

Figure 3. The proposed structure of the O-antigen PS of *E. coli* O152.

NMR

NMR spectra for the PS were recorded using Varian 400 and 600 MHz and Bruker 400 MHz instruments. NMR experiments performed were ^{31}P (1D), ^{13}C (1D), ^{13}C-DEPT (1D), ^{1}H (1D), ^{1}H,^{1}H-DQF-COSY, ^{1}H,^{1}H-TOCSY (mixing times of 30, 60 and 90 ms), ^{1}H,^{13}C-gHSQC, ^{1}H,^{13}C-gHMBC, ^{1}H,^{13}C-gHSQC-TOCSY (mixing times of 20 and 50 ms) and ^{1}H,^{31}P-COSY.

Acknowledgement

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