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Structural and immunological characterization of a glycoconjugate based on the delipidated lipopolysaccharide from a nontypeable *Helicobacter pylori* strain PJ1 containing an extended D-*glycero*-D-*manno*-heptan

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ABSTRACT

Structural characterization of the lipopolysaccharide (LPS) from a nontypeable *Helicobacter pylori* strain PJ1 and two corresponding mutants, PJ1 HP1283:cam and PJ1 HP1284:cam, was performed using a combination of NMR and mass spectrometric techniques. It resulted in the core structure that differed significantly from the one proposed previously. Overall architecture of PJ1 LPS was found to be consistent with a structural model described for several other *H. pylori* strains. It contained a polymer of D-*glycero*-D-*manno*-heptose (DD-Hep) as the O-chain component, linked to α-1,6-glucan through a DD-Hep oligosaccharide. *H. pylori* PJ1 HP1283:cam LPS was missing DD-heptan, terminating with an α-1,6-glucan chain containing 5–13 glucose residues. LPS of strain PJ1 HP1284:cam was missing DD-Hep from the core and had β-GlcNAc attached directly to O-3 of the inner-core LD-Hep residue. To investigate the role of DD-heptan in protective immunity, delipidated LPS (dLPS) from strain PJ1 was conjugated to tetanus toxoid (TT) and immunological properties of the resultant glycoconjugate dLPS(PJ1)-TT determined. The dLPS(PJ1)-TT conjugate was immunogenic in mice and rabbits and induced specific and cross-reactive functional antibodies against homologous and heterologous strains of *H. pylori*. Whole cell indirect ELISA performed on a selected number of *H. pylori* isolates confirmed that the immune response correlated with the presence of α-1,6-glucan and was not augmented by the DD-Hep content of these strains.

1. Introduction

Helicobacter pylori infections are associated with the development of chronic gastritis, peptic ulcer and gastric carcinoma and increased relative risk for gastric cancer [1].

We have previously demonstrated that synthetic glycoconjugates based on a truncated *H. pylori* lipopolysaccharide (LPS) devoid of Lewis antigens and containing an α-1,6-glucan in the outer core region induced broadly cross-reactive bactericidal antibodies and confer partial protection in a mouse model of *H. pylori* infection [2]. In the present study, we sought to investigate the candidacy of a glycoconjugate based on delipidated *H. pylori* LPS containing a long-chain D-*glycero*-D-*manno*-heptan. We rationalized that the vaccine strain comprising DD-heptan, in addition to α-1,6-glucan, could enhance the immunogenicity of a glycoconjugate and improve the vaccine efficacy. We prepared glycoconjugates based on delipidated LPS from a nontypeable *H. pylori* strain PJ1 previously reported to contain a long DD-heptan [3]. During the course of this investigation, the structure of *H. pylori* strain PJ1 LPS was revised and found to be identical to that of *H. pylori* strain D4 [4].

2. Results and discussion

H. pylori strain PJ1 was cultivated in liquid culture as previously described [5] and the LPS was extracted from the air-dried cells by hot phenol-water extraction procedure of Westphal and Jann [6]. Purified LPS was obtained from the aqueous phase following extensive dialysis and lyophilization, followed by ultracentrifugation.

Sugar analysis of the purified LPS from *H. pylori* strain PJ1 as alditol acetates revealed the presence of fucose (Fuc), glucose (Glc), galactose (Gal), *N*-acetylglucosamine (GlcNAc), D-*glycero*-D-*manno*-

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heptose (DD-Hep), and L-*glycero*-D-*manno*-heptose (LD-Hep) in the approximate molar ratio of 0.4: 3.0: 0.1: 1.3: 25.0: 1.0. In addition, methylation analysis of the LPS showed the presence of terminal and 3-substituted Fuc, terminal, 3- and 6-substituted Glc, terminal and 4-substituted Gal, terminal, 2-, 3-, 6-, 7-, 2,7- and 3,7-disubstituted DD-Hep, 2- and 3-substituted Hep and terminal-, 3- and 4-substituted GlcNAc.

Structural analysis of LPS from *H. pylori* strain PJ1 and two derived mutant strains was performed employing the same methods that were used for the analysis of other *H. pylori* strains [7]. Oligo - and poly-saccharides shown in Fig. 1 were obtained as follows:

A. Mild acetic acid hydrolysis of LPS with subsequent fractionation of the products by gel chromatography and anion-exchange chromatography.
B. Complete deacylation of LPS with 4 M NaOH followed by fractionation of the products by gel chromatography and HPAEC. This treatment causes removal of EtN from PEtN substituent at O-7 of Hep E and equal distribution of remaining phosphate between O-6 and O-7 of this monosaccharide, resulting in doubling of the signals of surrounding components.
C. Deamination of the products obtained according to procedure B.

All compounds were analyzed by 2D NMR (gCOSY, TOCSY, NOESY or ROESY, ^{1}H-^{13}C gHSQC, ^{1}H-^{13}C gHMBC, ^{1}H-^{13}C gHSQC-TOCSY, ^{1}H-^{31}P gHSQC). The assignment of NMR signals is shown in Table 1, Tables S1 and S2.

Polymer OS1 contained repeating units (OPS) built up of three DD-Hep residues. It was identical to the previously described polysaccharide from *H. pylori* strain D4 [4].

Typical *H. pylori* core and glucan were present in PJ1 LPS, but a terminal α-Glc-4-β-Gal-disaccharide was missing in all compounds analyzed here. An unusual feature of PJ1 LPS was the presence of a terminal heptose residue XX linked to O-7 of some heptose residues located in the post-glucan part of LPS. It was not present in lower molecular mass products and in HP1283:cam mutant, which had LPS ending at glucan. Signals of CH_2O group substituted with heptose XX did not overlap with any other signals and were easily identified at δ 3.61; 3.82/69.9 ppm. These signals belonged to a heptose residue (there are no other sugars beyond glucan), but it was unclear whether this heptose was a part of OPS or a linker region between glucan and OPS.

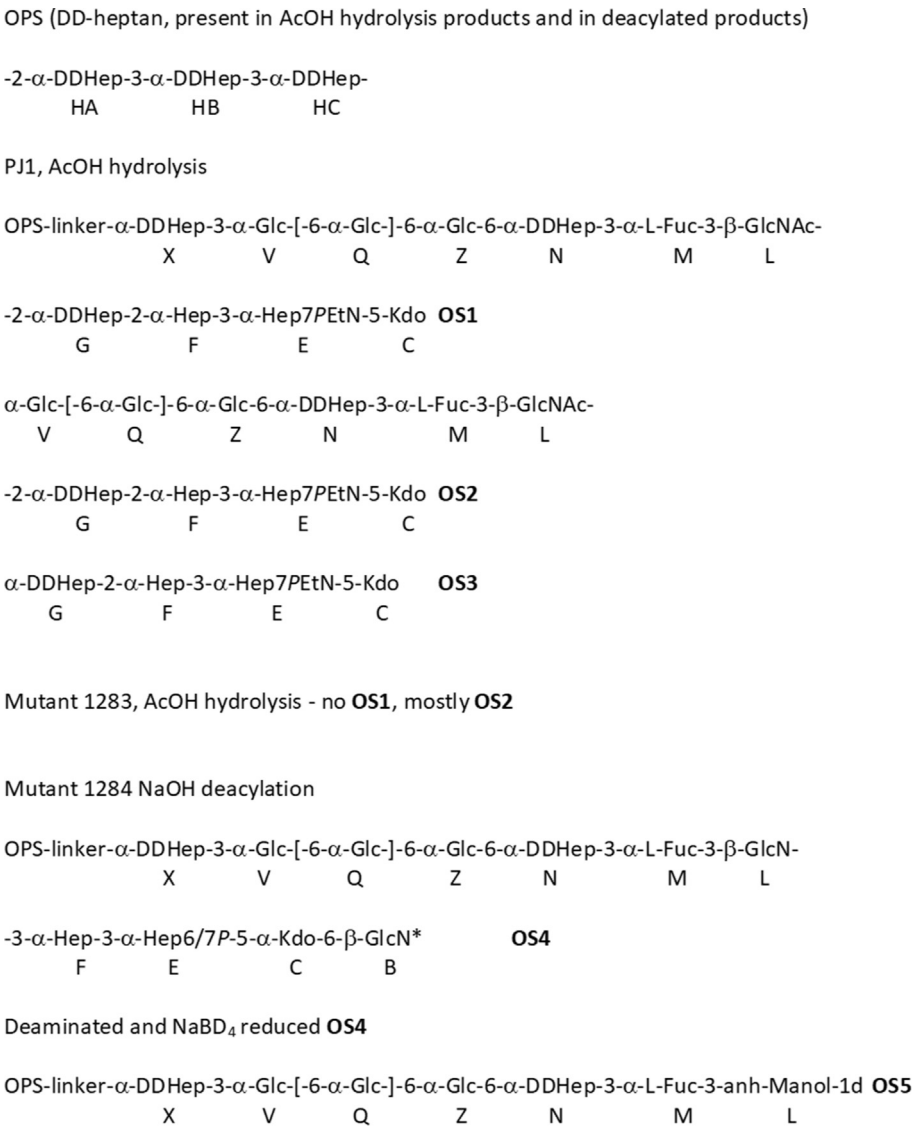

Fig. 1. Structures of the carbohydrate products obtained from LPS of *H. pylori* PJ1 and its mutants 1283 and 1284. *P*EtN indicates 2-aminoethyl phosphate, anhManol indicates 2,5-anhydromannitol (from GlcN). β-GlcN* B in OS4 is linked to degradation products of reducing terminal α-GlcN.