Carbohydrate Research 349 (2012) 90–94

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Note

Structural analysis of capsular polysaccharides expressed by *Burkholderia mallei* and *Burkholderia pseudomallei*

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ARTICLE INFO

Article history:
Received 4 November 2011
Received in revised form 13 December 2011
Accepted 14 December 2011
Available online 23 December 2011

Keywords:
Burkholderia mallei
Burkholderia pseudomallei
Capsular polysaccharide
Protective antigen
NMR
Structure

ABSTRACT

Capsular polysaccharides (CPSs) were isolated from O-polysaccharide deficient strains of *Burkholderia mallei* and *Burkholderia pseudomallei* using a modified hot phenol/water extraction procedure. Glycosyl composition, methylation, MALDI-TOF MS analyses as well as ^{1}H NMR spectroscopy including COSY, TOCSY, NOESY, HMBC and HSQC experiments identified the presence of two distinct CPS antigens in the samples exhibiting the following structures:

→3)-2-*O*-Ac-β-D-6dHep*p*-(1→

→3)-α-D-Man*p*-(1→

This study confirms the ability of *B. mallei* to express a 6-deoxy-heptan CPS and represents the first report of a mannan CPS being expressed by these bacterial pathogens.

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Burkholderia mallei is a non-motile, facultative intracellular, Gram-negative bacillus that causes a debilitating disease known as glanders. This zoonotic pathogen is an obligate animal parasite that is primarily responsible for disease in solipeds.[1–4] In equines, glanders presents as chronic or acute illnesses characterized by pulmonary involvement, ulcerative nasal/tracheal lesions and visceral abscess formation. The clinical progression of human glanders is similar to that observed in solipeds and may manifest as chronic or acute localized infections, acute pulmonary infections or fulminating septicemias.[1–3,5] Diagnosis and treatment of glanders can be challenging, and in the absence of chemotherapeutic intervention, disease is invariably fatal.[5–7] Due to the high risk of aerosol infection and the fact that this organism has previously been employed as an agent of biological warfare, *B. mallei* is currently listed as a select agent by the Centers for Disease Control and is recognized by the National Institute of Allergy and Infectious Diseases as a Category B Priority Pathogen.[8,9] Although glanders is one of the oldest of known infectious diseases, there are no human or veterinary vaccines available for immunization against this emerging infectious disease.[6]

Previous studies have demonstrated that *B. mallei* expresses a number of important virulence determinants that are required for survival in a variety of animal models of infection; included among these are an animal pathogen-like type III secretion system, the cluster 1 type VI secretion system and lipopolysaccharide (LPS).[10–12] Additionally, studies have shown that *B. mallei* expresses a capsular polysaccharide (CPS) that is both a virulence determinant and a protective antigen.[13,14] Although evidence suggests that this CPS is similar to the 1,3-linked 2-*O*-acetyl-6-deoxy-β-D-*manno*-heptan expressed by *Burkholderia pseudomallei*, the etiologic agent of melioidosis, the structure of the *B. mallei* antigen has yet to be solved.[13,15,16] In this report, we describe for the first time the structures of two CPS antigens expressed by *B. mallei* and demonstrate that both are also produced by *B. pseudomallei*.

Consistent with previous observations, immunoblot analyses demonstrated that *B. mallei* ATCC 23344 expresses a carbohydrate moiety that reacts strongly with the *B. pseudomallei* CPS-specific mAb, MCA147.[17] In contrast to the 6-deoxyheptan CPS expressed by *B. pseudomallei* 1026b, however, the molecular weight of the *B. mallei* antigen was observably lower (Fig. 1). To facilitate a more detailed analysis of CPS species expressed by *B. mallei*, CPS antigens were isolated from the O-polysaccharide (O-PS) deficient strain, BM1987, using a modified hot phenol/water extraction procedure.[15,18]

Glycosyl composition analysis of the resulting *B. mallei* CPS sample by GC–MS of the TMS-methyl glycosides detected mannose and a later eluting, initially unidentified monosaccharide in a 43:57 ratio. The identity of the latter monosaccharide was determined by methylation analysis, MALDI-TOF MS, and NMR. GC–MS of the partially

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doi:10.1016/j.carres.2011.12.011

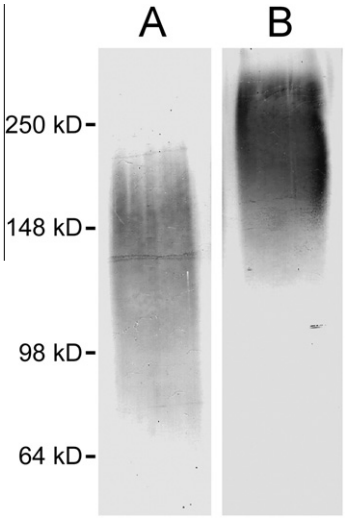

Figure 1. Western immunoblot analysis of CPS antigens expressed by (A) *B. mallei* ATCC 23344 and (B) *B. pseudomallei* 1026b. Proteinase K-treated whole cell lysates were probed with the *B. pseudomallei* CPS-specific mAb, MCA147.

methylated alditol acetates (PMAAs) of the sample showed two major peaks. The retention time and fragmentation pattern of the first peak was consistent with 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylmannitol-**1d** arising from 1,3-linked mannopyranose. The mass spectrum of the second peak had major ions at m/z = 118, 175, and 234. This fragmentation pattern is consistent with 1,3,5-tri-*O*-acetyl-6-deoxy-2,4,7-tri-*O*-methylmannoheptitol-**1d** or 1,3,5-tri-*O*-acetyl-7-deoxy-2,4,6-tri-*O*-methylmannoheptitol-**1d**, corresponding to 1,3-linked 6- or 7-deoxyheptopyranose. MALDI-TOF MS of the PMAAs also identified the deoxysugar as deoxyheptose by the presence of a peak with m/z = 388 (M+Na ion). The methylation analysis also showed several minor species arising from terminal, 1,4-linked, 1,3,6-linked, and 1,2,6-linked mannopyranose, terminal and 1,4-linked glucopyranose, terminal galactopyranose, terminal heptopyranose, and terminal, 1,2,3-linked, and 1,3,(6 or 7)-linked deoxyheptopyranose.

The 1-D proton NMR spectrum showed a rather complex anomeric region with three major peaks and several minor peaks, including shoulders on two of the major peaks (Fig. 2). The spectrum also displayed a carbohydrate ring region and several upfield signals indicating the presence of acetyl groups and one or more deoxysugar residues. COSY and TOCSY indicated that the three major signals in the anomeric region belonged to two separate spin systems. The HSQC spectrum revealed that the most downfield peak (5.29 ppm) in this region was not an anomeric proton, but H-2 of a residue acetylated on *O*-2. Tracing the cross peaks of this spin system in the COSY spectrum showed that it contained a deoxymethylene group next to an oxygenated methylene group. HSQC determined that C-3 of this residue was at 81.6 ppm, indicating glycosylation in this position. Together with the results from methylation analysis, this data demonstrated that this residue was a 1,3-linked 2-*O*-acetyl-6-deoxyheptopyranose. Comparison of chemical shifts with results published on the LPS of *B. pseudomallei* demonstrated that it was identical to the 1,3-linked 2-*O*-acetyl-6-deoxy-β-D-*manno*-heptopyranose described therein (Table 1),[16] and GC–MS of TMS-2-butyl glycosides confirmed that both have the same absolute configuration (D).

The chemical shifts of the other major spin systems were consistent with 3-linked α-mannose. NOESY gave a strong cross peak between α-Man H-1 and α-Man H-3 (Fig. 3). Since the geometry of α-Man precludes an intra-residue correlation between H-1 and H-3, this must be an inter-residue correlation. Neither NOESY nor HMBC revealed a correlation between α-Man and the deoxyheptose residue (Figs. 3 and 4). Together, these results suggest that the two residues were not part of the same polysaccharide, but that the sample consisted of a mixture of a 1,3-linked 2-*O*-acetyl-6-deoxy-β-D-*manno*-heptan and a 1,3-linked α-mannan (Table 1). Integration of the anomeric signals of the mannan and the deoxyheptan in the 1-D ^{1}H spectrum gave a ratio of 48:52. From minor COSY cross peaks adjacent to the cross peaks between H-1 and H-2 and between H-2 and H-3 of the acetylated residue it was

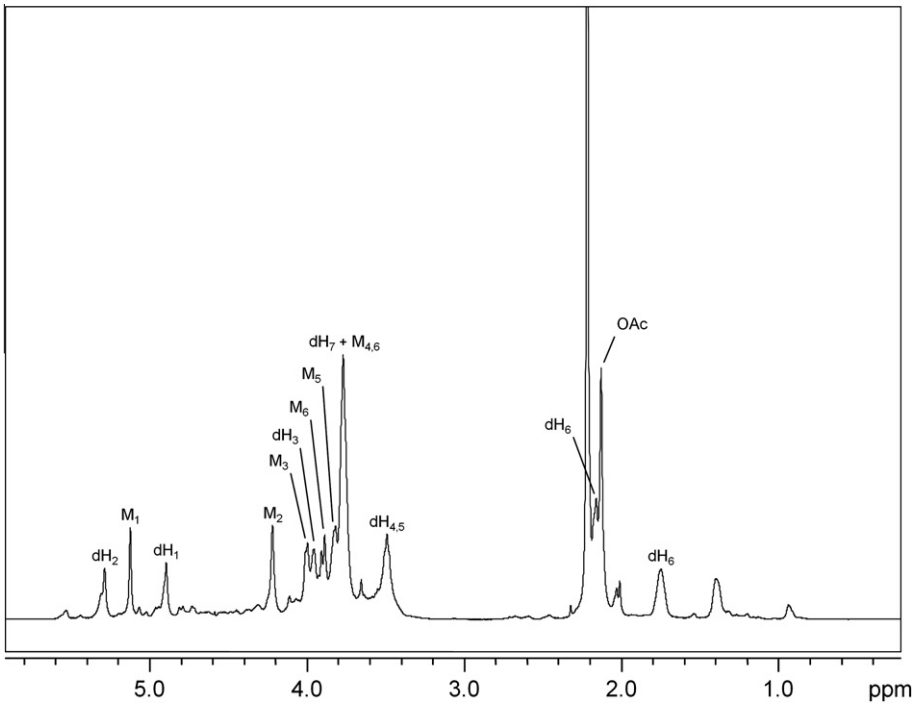

Figure 2. 1D ^{1}H NMR spectrum of the CPS antigens isolated from *B. mallei* BM1987. dH, 6-deoxy-heptose; M, mannose; OAc, *O*-acetyl.

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