

Identification of a new monosaccharide in *Mycobacterium tuberculosis* lipoarabinomannan

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Introduction

Tuberculosis (TB) remains a major threat to world health, with approximately eight million cases of TB resulting in two million deaths per annum. Ninety-five percent of TB cases arise in the developing world, where the HIV epidemic has dramatically increased the number of people who succumb to the disease. The WHO has highlighted the urgent need for more effective anti-TB drugs and for rapid, specific and sensitive diagnostic tests for the causative agent, *Mycobacterium tuberculosis* (Mtb). The ability of the Mtb to enter and colonise macrophage cells can, in part, be attributed to a carbohydrate structure called lipoarabinomannan (LAM, Fig. 1a), that is present in the Mtb cell wall. Mannose residues that cap the dendritic arabinan chains of LAM, facilitate entry of Mtb into alveolar macrophages, following interaction with the macrophage mannose receptor. LAM then promotes the intracellular survival of Mtb by down-regulating the immune response and providing anti-oxidative protection for the bacterium. The mechanisms by which LAM exerts its biological activity inside macrophage cells remain to be elucidated.

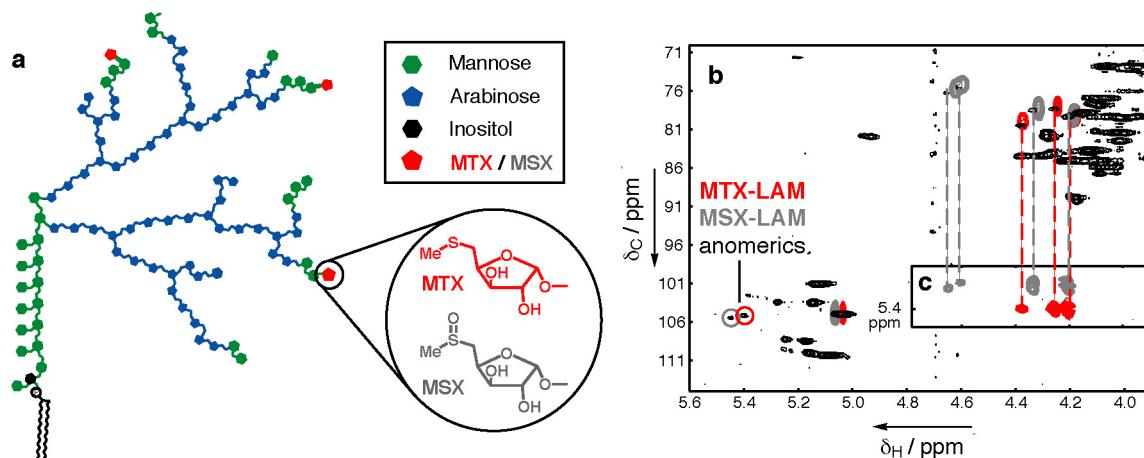


Fig. 1. (a) Cartoon representation of LAM showing the location of MTX/MSX (red) attached to the mannosyl caps (green) at the ends of the arabinan (blue) chains. (b) An overlay of HSQC spectra for the α -methyl glycosides of MTX (red), and MSX (grey) and LAM (black). (c) Inset is a strip from an HCCH-TOCSY spectrum of LAM which highlights the ^1H chemical shifts for MTX and MSX that correlate with the anomeric signals at ca. 5.4 ppm

Recently, we discovered an unusual sugar residue attached to the mannosyl caps of LAM. High resolution NMR spectroscopy and mass spectrometry studies revealed that this novel monosaccharide was a 5-methylthio-pentofuranoside, which was partially oxidised to the sulfoxide. However, it was not possible to determine the stereochemistry of the pentose using NMR methods. Consequently, we chemically synthesised all eight possible isomers of methylthio-pentose in the form of their methyl glycosides. On comparing the HSQC spectra for these compounds with that for LAM, it became clear that only the α -xylo-configured sugar (methylthio-xylofuranoside, MTX) gave signals that correlated with the natural product (Fig. 1b and c). Similarly, the chemical shifts for the sulfoxide form of the natural sugar were in generally good agreement with those for the oxidised α -xylofuranoside (MSX).

The discovery of MTX constitutes the first example of a methylthio-sugar residue incorporated into a polysaccharide, and only the fifth example of a xylo-configured sugar

outside the plant kingdom. Presumably, the biosynthetic precursor of MTX is methylthioadenosine – a ubiquitous by-product of polyamine biosynthesis that is derived from SAM. Nevertheless, Mtb invests significant biosynthetic effort into incorporating MTX into its cell wall, which implies that this sugar may provide some advantage to the bacterium. The occurrence of MTX at the non-reducing termini of the polysaccharide, locates it at the bacterium's surface, where it could easily act as a ligand for receptors or regulatory proteins, either on the surface of the macrophage, or inside the host cell, following endocytosis. It is possible that conversion of MTX to its oxidised form (MSX) may even play a role in LAM-mediated oxidative protection for Mtb. Further structural and biochemical studies of MTX are in progress.

Collaborators

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Publications

Turnbull, W.B., Shimizu, K.H., Chatterjee, D., Homans, S.W. and Treumann, A. (2004) Identification of the 5-methylthiopentosyl substituent in *Mycobacterium tuberculosis* lipoarabinomannan. *Angew. Chem. Int. Ed.* **43**, 3918-3922.

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