Structural and enzymatic studies on a novel substituent of *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. 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 lipid-anchored polysaccharide, lipoarabinomannan (LAM, Fig 1), is a major component of the Mtb cell wall, and an important virulence factor for the bacterium. 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.

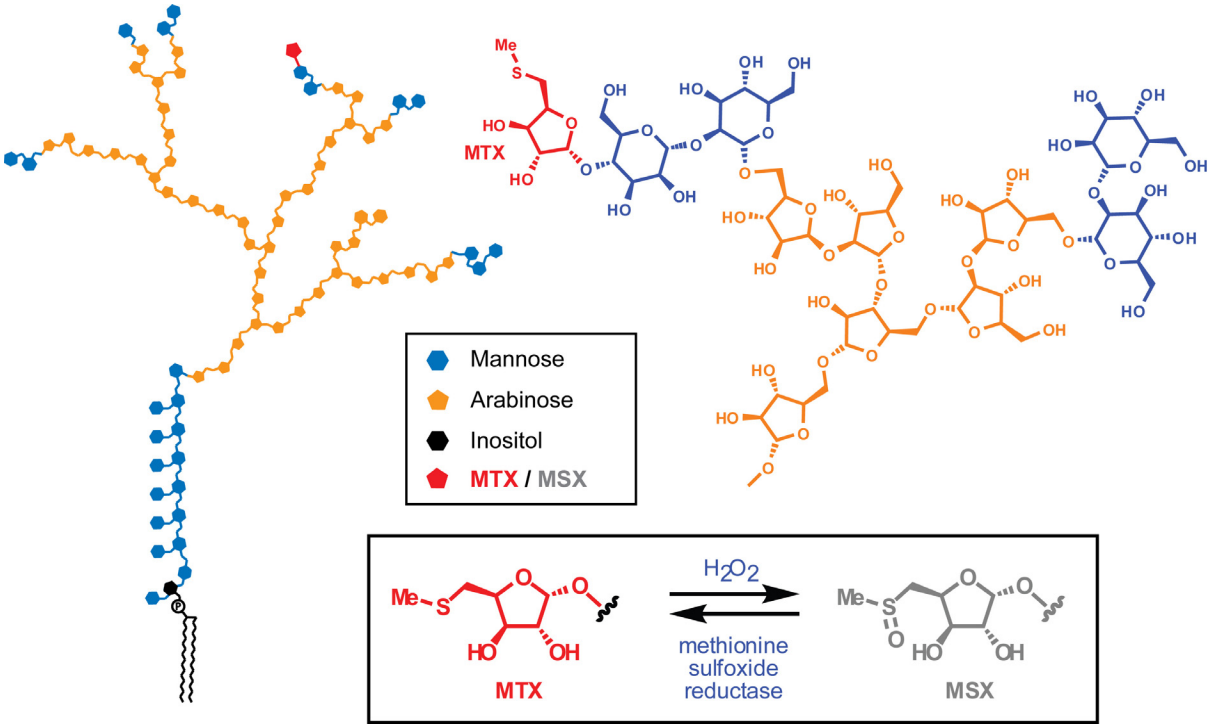

Figure 1. Schematic representation of LAM showing the structures of the MTX and MSX substituents and their interconversion by chemical oxidation and enzymatic reduction.

Recently, we discovered an unusual methylthiopentosyl residue attached to the mannosyl caps of LAM, which we subsequently identified as having an α-xylo configuration. This unusual discovery constitutes the first report of a methylthio-sugar residue incorporated into a polysaccharide, and one of very few examples of a xylo-configured sugar outside the plant kingdom. 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 concurrent discovery of an oxidised form of the sugar (methylsulfinylxylose, MSX, Fig. 1), implies that MTX may play a role in oxidative protection for Mtb.

We have demonstrated that exposure of LAM to the biological oxidant H_2O_2 results in oxidation of only the MTX substituent (Fig.2). We have also found that the *S*-configured MSX sulfoxide can be reduced by the mycobacterial methionine sulfoxide reductase enzyme (MsrA). While oxidation of MTX would almost certainly affect its biological function *in vivo*, this damage could

be repaired, in part, by MsrA which is present in the mycobacterial cell wall/membrane fraction. Alternatively, there exists the possibilty that MSX on the surface of *M. tuberculosis* may be reduced by host MsrA (and MsrB that reduces the *R*-sulfoxide) when the bacteria reside inside macrophages. If so, then MSX would be the first natural non-protein substrate for these enzymes. Furthermore, this redox cycle of chemical oxidation and enzymatic reduction could also provide a mechanism for more general anti-oxidative protection, as has been established previously for methionine oxidation. Indeed, as H_2O_2 is a direct precursor of hydroxyl radicals in vivo, sequestration of H_2O_2 by MTX/MsrA could also reduce the production of OH• in the mycobacterial cell wall. The MsrA-catalysed reduction of MSX-LAM has also allowed us to prove that the MSX/MTX sugars have the absolute D-configuration.

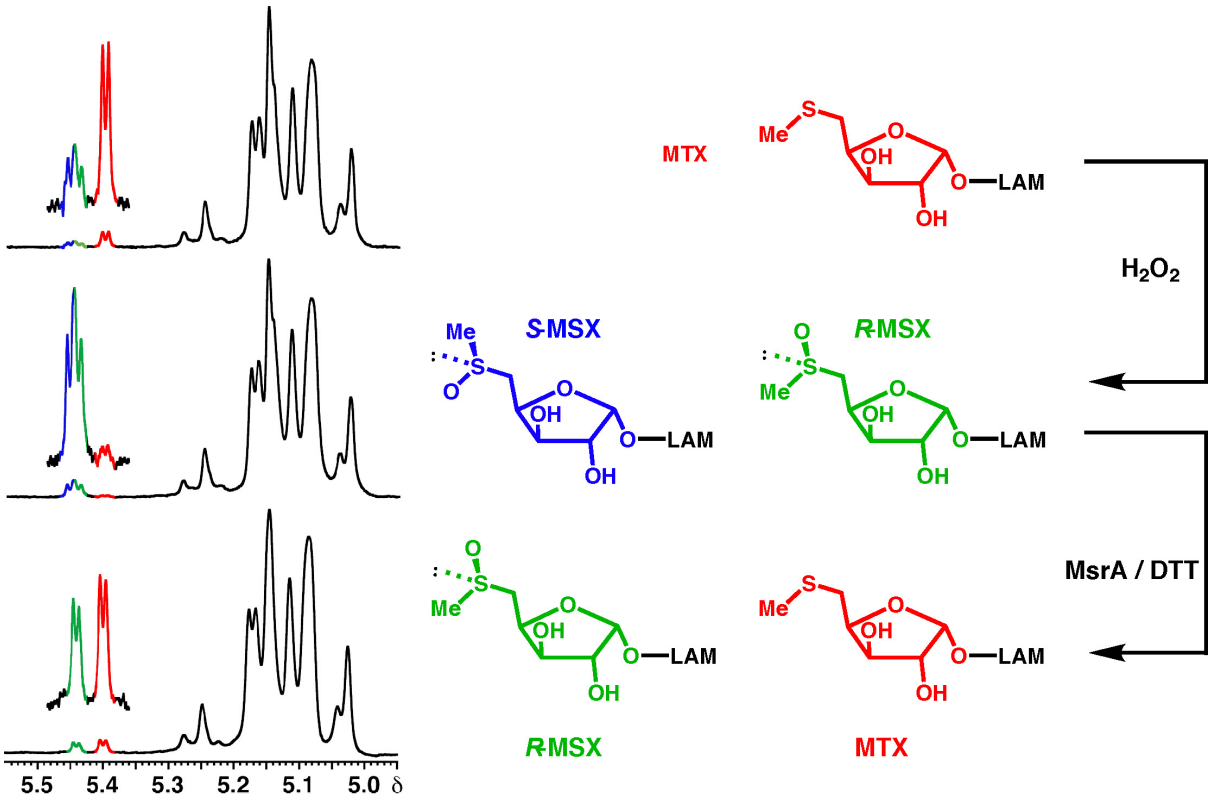

Figure 2. NMR spectra of *M. tuberculosis* LAM with the MTX and MSX anomeric protons inset. Oxidation of MTX leads to two diastereoisomers of MSX, one of which is reduced back to MTX using the MsrA enzyme.

Publications

Stalford, S.A., Fascione, M.A., Sasindran, S.J., Chatterjee, D., Dhandayuthapani, S. & Turnbull, W.B. (2009) A natural carbohydrate substrate for *Mycobacterium tuberculosis* methionine sulfoxide reductase A, *Chem. Commun.*, 110-112.

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