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## Identification of the sugars involved in mycobacterial cell aggregation

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### Abstract

Incubation of *Mycobacterium tuberculosis* and *M. smegmatis* cells with the sugar components of their surface-exposed glycans demonstrated that D-arabinose, but not  $\alpha$ -D-glucose or D-mannose, led to the dispersion of the large clumps formed by the bacilli in stationary liquid cultures. These results confirm the presence of arabinose-containing glycans on the mycobacterial cell surface and demonstrate the implication of selective sugars in cell aggregation, suggesting that the clumping of mycobacterial cells is probably mediated by lectin-carbohydrate interactions.

**Keywords:** Mycobacteria; Tuberculosis; Cell aggregation; Carbohydrates

### 1. Introduction

Mycobacteria are Gram-positive, acid-fast bacteria possessing a cell envelope composed of highly characteristic constituents [1]. The tubercle bacillus and most other species of the genus *Mycobacterium* are known to form large clumps, especially in stationary liquid culture. This moldlike growth pattern of mycobacteria hampers conventional in vitro studies such as the determination of growth curves by turbidimetry, drug testing and experimental infection of macrophages. To achieve these purposes, methods are currently used, however, that may damage the bacilli and/or extract part of the outermost surface-exposed material from the cells. Although the chemi-

cal basis of mycobacterial cell aggregation is not known, it is assumed that the postulated large amounts of surface-exposed lipids render the mycobacterial surface hydrophobic and consequently facilitate seeding for optimum surface cultivation of these obligate aerobes [1]. We have recently defined the mycobacterial outermost cell envelope constituents as being composed mainly of polysaccharides and proteins, with only small amounts of lipids [2,3]. These data obviously question the implication of lipids in mycobacterial cell aggregation and raise the possibility of the involvement of protein-protein and/or carbohydrate-protein interactions in this phenomenon. The present study investigates the implication of the major surface-exposed glycans of *M. tuberculosis* and most other non-tuberculous mycobacterial species, i.e., the glycogen-like glucan, arabinomannan, and mannan [2,3], in cell clumping. In

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addition, xylose-containing glycans were also identified in some species such as *M. smegmatis* [3]. Consequently, the sugar constituents of these glycans and xylose were used to inhibit the clump formation that naturally occurs during mycobacterial growth.

## 2. Materials and methods

*Mycobacterium tuberculosis* H37Rv (ATCC 27294) and *M. smegmatis* (ATCC 607) were grown on Sauton's medium (100 or 30 ml per flask) as surface pellicles at 37°C for 3 weeks and 3 days, respectively. The sugar to be tested was then added to the flasks at a final concentration of 1 or 10 mg ml<sup>-1</sup> and the cultures were shaken at 37°C for 16 h. In the control experiments, the mycobacterial cultures were shaken overnight with no addition of sugar.

D-Allose, D- and L-arabinose, 2-deoxy-D-glucose, D-galactose, D-glucose, inositol, D-mannose,  $\alpha$ - and  $\beta$ -D-methylglucoside, and D-xylose were dissolved in distilled water (30, 100, 300 or 1000 mg ml<sup>-1</sup>) and sterilized. 1 ml of the carbohydrate solution was added to sterile fresh Sauton's medium.

## 3. Results

### 3.1. Declumping of *M. smegmatis* cells

Table 1 shows the effects of the addition of various

sugars (at a concentration of 1 mg ml<sup>-1</sup>) on the aggregation of *M. smegmatis* cells. Not all the sugars were efficient in inducing the declumping of aggregated cells, demonstrating the specificity of the reaction. For instance, the addition of D-arabinose and D-xylose, but not L-arabinose, led to the observation of fine particles in the shaken cultures as opposed to the large clumps formed in control cultures. Among the hexoses tested, D-glucose, but not D-mannose, also induced a declumping of cells similar to that observed with D-arabinose. The addition of 10 mg ml<sup>-1</sup> of mannose, 10 times the concentration in the standard experiments, did not reverse cell agglutination. In agreement with the negative result obtained with mannose, 2-deoxy-D-glucose was also inefficient in inhibiting cell aggregation, demonstrating the crucial role of the configuration of carbon 2 in the reaction. To further analyze the importance of the configuration of the other carbons of the pyranosyl ring, D-allose and D-galactose, the epimers of D-glucose on positions 3 and 4 respectively were tested. These two sugars were not efficient in inhibiting cell clumping, even when large amounts (10-fold that of glucose) were added to the cultures, showing that both positions are important for the inhibition of cell aggregation. In contrast, position 6 was not important for the inhibition, since inositol was as efficient as glucose in inducing the declumping of cells. The contribution of the anomeric position in the glucosyl ring was investigated by comparing the effects of the two methyl glycosides on the cell aggre-

Table 1

Effects of the addition of various carbohydrates on the aggregation of mycobacterial cells<sup>a</sup>

Compound	<i>Mycobacterium smegmatis</i>	<i>Mycobacterium tuberculosis</i>
None	—	—
D-Allose	—	nt
D-Arabinose	+	+
L-Arabinose	—	nt
D-Galactose	—	nt
D-Glucose	+	—
$\alpha$ -Methyl-D-glucoside	—	nt
$\beta$ -Methyl-D-glucoside	+	nt
2-Deoxy-D-glucose	—	nt
Inositol	+	nt
D-Mannose	—	—
D-Xylose	+	—

<sup>a</sup>To mid-log cultures of *M. smegmatis* and *M. tuberculosis*, 1 mg ml<sup>-1</sup> of carbohydrate (final concentration) was added and the flasks were shaken for 16 h. +: declumping of the cell aggregates; —: no inhibition of cell clumping; nt: not tested.

gation (Table 1). The  $\beta$  anomer of glucose, but not the  $\alpha$  one, induced the declumping of cells.

### 3.2. Declumping of *M. tuberculosis* cells

The sugar components of the surface-exposed glycans of the tubercle bacillus were tested for the inhibition of the cell aggregation (Table 1). D-Arabinose was efficient in inhibiting the formation of large clumps in shaken cultures, a result consistent with the data obtained on *M. smegmatis*. Conversely, the addition of D-xylose to the tubercle bacilli did not induce the dispersion of clumps, a result that is consistent with the tiny amount of xylose (2%) present in the acid hydrolysis products of the surface-exposed material of *M. tuberculosis* [2]. Surprisingly, although glucose represents the major constituent of the surface-exposed glycans of the tubercle bacillus [2], the addition of this sugar did not induce the declumping of cells.

## 4. Discussion

The present study was undertaken in order to determine the implication of carbohydrates in mycobacterial cell aggregation, a moldlike growth pattern that typifies these organisms, currently assumed to be due to lipid-lipid interactions [1]. The recent demonstration of the presence of large amounts of glycans on the cell surface of mycobacteria [2,3] led us to reconsider the proposed concept. The addition of various sugars that compose the surface-exposed glycans of *M. tuberculosis* and *M. smegmatis* resulted in the identification of D-arabinose as the sugar that inhibits cell aggregation in both species. Thus, the corresponding surface-exposed arabinose-containing polysaccharides, i.e., arabinomannans [2], and the xylose-containing glycans (xylans) are probably involved in the aggregation of mycobacterial cells. These results, in turn, confirm the presence of large amounts of carbohydrates on the cell surface of mycobacteria. The demonstration that carbohydrates are involved in cell clumping and the identification of the sugar constituents participating in this phenomenon may help in obtaining the declumping of cells. In the case of *M. smegmatis*, it also appeared that D-xylose and  $\beta$ -glucose, but not  $\alpha$ -glucose, abol-

ish cell clumping. This result indicates that the glycogen-like glucan, which is entirely composed of  $\alpha$ -glucosyl units, is not involved in cell aggregation. One possible explanation of the efficiency of the  $\beta$  anomer of glucose lies in the structural analogy between the xylopyranosyl and the glucopyranosyl residues. This assumption was based on the fact that xylose is known to exist predominantly in the  $\beta$ -pyranosyl form in solution; thus, both sugars share a similar configuration for carbons 2–5. As position 6 is not important for the inhibition, the two pyranosyl forms exhibit a strong similarity. This hypothesis is consistent with the fact that xylose occurs in the mycobacterial surface-exposed xylans in the  $\beta$ -xylopyranosyl form (unpublished results of A. Ortalo-Magné and M. Daffé).

During the past decade, research related to interactions involving carbohydrates has grown in size and scope. This has resulted in the isolation and characterization of a class of proteins, the so-called lectins, not only from plants and animals, but also from bacteria [4]. In the context of the present study, the obvious partners of the mycobacterial carbohydrates involved in cell aggregation are also lectins, the presence of which has been reported [5,6]. A 12–14 kDa lectin has been purified from the extracellular material of *M. smegmatis* [5], tentatively characterized as a mannose-binding lectin and subsequently called mycotin [6]. Immunologically related molecules were also shown to be present in the walls of *M. avium* and *M. tuberculosis* [6]. In our hands, however, application of affinity chromatography using mannose-coupled agarose was unsuccessful for isolating any mannose-binding protein from the extracellular and surface-exposed materials of *M. tuberculosis* and *M. smegmatis*. Nevertheless, as the agglutination of erythrocytes induced by mycotin, as well as the adherence of mycobacteria to macrophages, is reversed or inhibited by yeast mannan [5,6], this lectin is probably not involved in cell aggregation which is not, as shown here, inhibited by mannose. Another potential candidate that may be involved in cell aggregation is the 30–31 kDa, so-called antigen 85 [7]. Proteins from this family bind to fibronectin and represent the main proteins of the culture filtrate and surface-exposed material of *M. tuberculosis* [2]. Furthermore, proteins belonging to the family of antigen 85 have been characterized in

the extracellular materials of all the mycobacterial species examined so far. When the lectin activity of the purified protein was checked by measuring the agglutination of human erythrocytes, however, no agglutination was observed (data not shown). Thus, further studies are needed in order to characterize the mycobacterial lectins involved in cell aggregation.

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