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Purification and characterization of an extracellular lectin from *Mycobacterium smegmatis*

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Mycobacteria exist naturally in aggregated form and pathogenic strains colonize macrophages. A lectin has been isolated from the culture broth of *M. smegmatis*, which may, possibly, have an important role in either or both of these phenomena. The lectin of M_r 12000–14000 agglutinates erythrocytes from different species, and agglutination is reversed by arabinogalactan isolated from mycobacteria, as well as by yeast mannan. It has a p*I* of 5.5 and is rich in aspartic and glutamic acid residues.

Lectin, extracelllular; Arabinogalactan; Mannan; (*Mycobacterium smegmatis*)

1. INTRODUCTION

In recent years considerable interest has been generated in the characterization of microbial agglutinins, since many of these play a role in mediating adherence to surfaces colonized by the microorganism [1–3] and many serve as recognition determinants during development [4,5]. The *Mycobacteria*, members of which genus cause leprosy and tuberculosis, have a cell surface that is rich in carbohydrates and extremely hydrophobic. The dominant carbohydrate-containing epitopes of the pathogenic *Mycobacteria* are the phenolic glycolipids, lipoarabinomannan and the arabinogalactan-peptidoglycan complex [6]. Since *Mycobacteria* exist naturally in aggregated form and pathogenic strains colonize macrophages, we speculated that lectin-carbohydrate interactions may play a role in either or both of these phenomena. In the present paper we report the characterization of an extracellular lectin from the culture broth of *Mycobacterium smegmatis*.

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2. MATERIALS AND METHODS

2.1. *Growth of organism*

Mycobacterium smegmatis SN2 was grown at 37°C in Youmans-Karlson's medium [7] containing the following (in g/l): 5.0 asparagine; 0.5 KH_2PO_4; 1.5 citric acid; 0.6 $MgCO_3$; and 20 ml glycerol, pH 7.0.

2.2. *Purification of lectin*

Protein was isolated from the supernatant of the culture broth of the mycobacteria grown for 66 h by precipitation with ammonium sulfate (52 g/100 ml). After stirring for 45 min, protein was precipitated by centrifugation at 10000 × *g* for 30 min at 4°C, dissolved in 5 mM sodium phosphate buffer (pH 7.2) containing 1 mM phenylmethylsulfonyl fluoride, and dialysed against the same. The dialysate was loaded on a DEAE-Sephadex column (1.6 × 30 cm) equilibrated against 5 mM sodium phosphate buffer (pH 7.2). A gradient of 0–0.3 M NaCl in the same buffer was used to elute bound proteins.

2.3. *Determination of protein*

Protein was estimated according to the method of Lowry et al. [8].

2.4. *Hemagglutination assay*

This was done by the two-fold serial dilution technique using a 1% suspension of rabbit erythrocytes. The inhibition of ag-

glutination with sugars was assayed according to Osawa and Matsumoto [9].

2.5. *Gel electrophoresis*

Non-denaturing gel electrophoresis was carried out at 4°C according to the method of Davis [10]. SDS-gel electrophoresis was carried out according to Laemmli [11].

2.6. *Gel filtration*

This was carried out on a Sephadex G-75 column (1.6 × 70 cm) calibrated with albumin, ovalbumin, chymotrypsinogen and ribonuclease.

2.7. *Isoelectric focusing*

This was carried out on 5% polyacrylamide gels using ampholines, pH 3.5–10, in an LKB Multiphor II apparatus at 25 W constant power for 2 h according to the manufacturers' instructions.

2.8. *Amino acid analysis*

The purified lectin was hydrolysed at 110°C with 6 N HCl for 24, 48 and 72 h. Amino acids were analysed in an LKB Alpha Plus amino acid analyzer.

2.9. *Isolation of cell wall carbohydrate*

Cell wall arabinogalactan was isolated from acetone-dried cells of *M. smegmatis* according to the method of Misaki and Yukawa [12]. Briefly, cells were hydrolysed with NaOH, neutralised and dialysed against water. Polysaccharides were fractionated from the undialysable fraction by graded ethanol precipitation. After washing with ethanol, polysaccharide fractions were dried under vacuum. The purity of the polysaccharides was tested by paper chromatography after acid hydrolysis. Purity was further checked by GLC of the monosaccharides after trimethylsilylation with Tri-Sil Z (Pierce Co.) as described by Clamp et al. [13]. Peaks were identified on a 3% SE-30 column using standard galactose and arabinose silylated as described above.

3. RESULTS AND DISCUSSION

Fractions showing hemagglutinating activity after elution from the DEAE-Sephadex column were pooled (fig.1). About 200 μg lectin was obtained from 4 liters of the culture broth. The pooled protein was homogeneous on electrophoresis in non-denaturing gels at pH 8.3.

3.1. *Biochemical properties*

SDS-gel electrophoresis of the purified protein gave a single subunit (fig.2). From the logarithmic calibration plot the M_r was found to be 14000. Gel filtration on Sephadex G-75 showed that the protein emerges as a single peak corresponding to an M_r of 12000, suggesting that it is a single chain polypeptide of M_r 12000–14000. The lectin was rich in glutamic acid, aspartic acid and alanine residues (table 1) and showed a p*I* of 5.5 (fig.3).

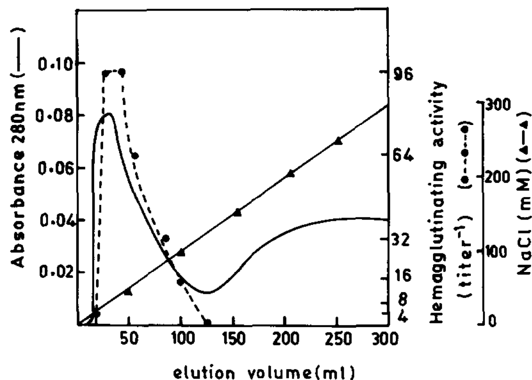

Fig.1. DEAE Sephadex chromatography of the culture broth after ammonium sulfate precipitation. After loading the protein, the column was washed with 5 mM phosphate buffer (pH 7.2). Elution of the adsorbed material with a linear gradient of 0–0.3 M NaCl is shown in the figure.

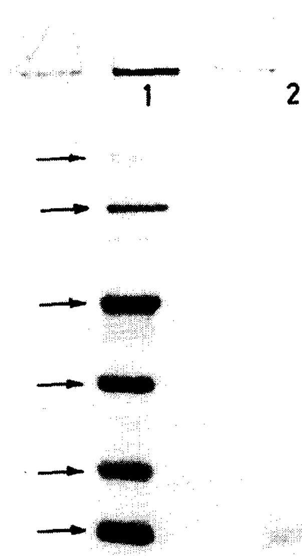

Fig.2. SDS-PAGE of the purified mycobacterial lectin. 1: Molecular weight markers, from the top: phosphorylase *b* (97400), bovine serum albumin (66200), egg albumin (42699), bovine carbonic anhydrase (31000), soybean trypsin inhibitor (21500) and hen egg white lysozyme (14400). 2: Mycobacterial lectin.

Table 1

Amino acid composition of the mycobacterial lectin

Amino acid	Residues/100 residues
Asx	12.4
Thr	7
Ser	5.5
Glx	12.7
Pro	6.2
Gly	9.3
Ala	12.2
Cys	–
Val	8.4
Met	0.23
Ile	4.4
Leu	7.6
Tyr	1.6
Phe	3.1
His	1.5
Lys	5.1
Arg	2.8
Trp	not determined

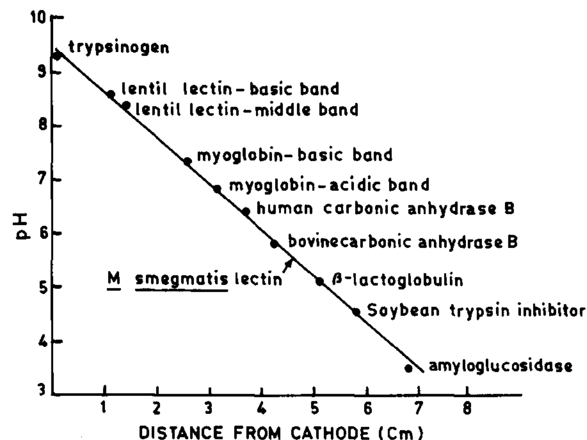

Fig.3. Isoelectric focusing of the mycobacterial lectin, using marker proteins with isoelectric points of 9.30 (trypsinogen), 8.65 (lentil lectin, basic band), 8.45 (lentil lectin, middle band), 7.35 (myoglobin, basic band), 6.85 (myoglobin, acidic band), 6.55 (human carbonic anhydrase B), 5.85 (bovine carbonic anhydrase B), 5.20 (β-lactoglobulin A), 4.55 (soybean trypsin inhibitor) and 3.50 (amyloglucosidase).

3.2. *Agglutination properties and inhibition by carbohydrates*

The lectin agglutinated erythrocytes from different animals (table 2). It agglutinated human A, B and O erythrocytes nonspecifically.

Table 3 summarizes the results of inhibition studies of agglutination of rabbit erythrocytes by different sugars. Simple monosaccharides could not inhibit lectin-mediated agglutination. D-Arabinose was effective at a concentration of 0.1 M. L-Arabinose was not inhibitory. Agglutination could not be reversed by fetuin, bovine submaxillary mucin or hog gastric mucin. Attempts were then made to determine whether agglutination could be reversed by carbohydrates which are unique constituents of the outer surface of *Mycobacteria*. Arabinogalactan purified from *M. smegmatis* was found to reverse agglutination. However, arabinogalactan isolated from larchwood did not show any inhibitory activity. This is interesting, since mycobacterial arabinogalactan contains arabinose in the D-form and both glycosyl residues in the unusual furanose form [14].

Agglutination was also reversed by yeast mannan. *p*-Nitrophenyl α-D-mannopyranoside was inhibitory, suggesting the importance of hydrophobic moieties in the interaction of the lectin with sugars. However, *p*-nitrophenyl β-D-mannopyranoside was not inhibitory.

Interest is now being centered on studying the role of lectins in bacterial adherence as well as colonisation of bacteria, involved in several disease mechanisms. In *Vibrio cholerae*, participation of fimbriae and hemagglutinins in motility has already been established [15]. The role of lectin in fundamental processes like development and morphogenesis has been studied in the slime mold, *Dictyostelium discoideum* [5]. Lectin-mediated cell-cell adhesion has been found to be an important determinant in the progress of its life cycle.

Table 2

Hemagglutinating activity of the mycobacterial lectin with erythrocytes from different animals

Animal	Number tested	Minimum hemagglutinating dose (μg/ml)
Rabbit	4	0.5
Cow	2	20
Goat	2	8
Chicken	2	2
Human A	2	3
Human B	2	3
Human O	2	3

Table 3

Inhibition of lectin-mediated hemagglutination

Sugar	Minimum amount inhibiting four hemagglutinating doses
D-Arabinose	0.1 M
Arabinogalactan from *M. smegmatis*	37.5 mg/ml
p-Nitrophenyl α-D-mannopyranoside	10 mM
Mannan (yeast)	2 mg/ml

Non-inhibitory (up to 0.2 M): D-glucose, D-galactose, L-fucose, D-xylose, L-rhamnose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, *N*-acetylneuraminic acid, *p*-nitrophenyl β-D-mannopyranoside, fetuin, bovine submaxillary mucin, hog gastric mucin

The specificity of the mycobacterial lectin for mycobacterial arabinogalactan as well as yeast mannan is quite unique, suggesting a role of the lectin in mycobacterial cell growth and, possibly, in pathogenicity.

It is tempting to speculate that the soluble lectin may be involved in forming a bridge between bacteria and phagocytes by binding to carbohydrate moieties present on the surface of both types of cells. Further studies are in progress to determine whether this lectin is present in pathogenic strains also, and if so, its possible implications in mycobacterial pathogenicity.

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