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Note

Some structural features of polysaccharide components of the protozoan *Leishmania tarentolae*

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Studies on the serological reactions of lizard *Leishmanias* and their antigenic relationships with human species of *Leishmanias* are few. To better the understanding of these relationships, it is important to study the surface components of different species of *Leishmanias*. Manson-Bahr and Southgate[1] reported the existence of a considerable degree of cross immunity in humans between *L. adleri* and *L. donovani*, and evidence of some degree of cross immunity between *L. adleri* and *L. braziliensis*, *L. enrietti*, *L. tarentolae*, and *L. oncopelti*. The results were in agreement with the serological studies of Adler[2]. He showed that these antigenic relationships provide further evidence to support the hypothesis that *L. adleri* and probably *L. hoogstraali* and *L. tarentolae* represent transitional stages in the evolution of *Leishmania* from a purely reptilian to a mammalian parasite.

Since it is likely that polysaccharides play a role in these shared antigenic properties, samples were isolated from *L. tarentolae* and analyzed chemically. This organism was chosen for the present study as it is readily cultured *in vitro*. It can also be grown in different media[3,4], a useful characteristic for studying multicomponent polysaccharide-systems in protozoa. For example, Mendonça-Previato *et al.*[5] and Gorin *et al.*[6] reported differences in the ratios of polysaccharides occurring in cells of *Herpetomonas samuelpessoai* and *Crithidia fasciculata*, grown in different media. The ratios were also influenced by age of culture.

Polysaccharides were isolated from cells grown on two media, designated A (ref. 7) and B (ref. 8) (see Experimental section), by extraction with hot alkali, purification by ethanol precipitation, and subsequent treatment with ion-exchange resins. The products are called, respectively, Polysaccharide A and B. As can be seen from Table I, the carbohydrate composition of both cells and isolated polysaccharides

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TABLE I

SUGAR COMPOSITION OF CELLS AND EXTRACTS OF *L. tarentolae*

Medium[a]	*Source*	*Component sugar (%)*					
		Rib	*Ara*	*Man*	*Gal*	*Glc*	*Inositol*
A	Whole cells	13		58	8	11	10
A	KOH Extract (polysaccharide A)		trace	68	14	6	12
B	Whole cells	27	trace	28	8	31	6
B	KOH Extract (polysaccharide B)		10	7	6	10	7
B	Glycoprotein		trace	60	16	17	7

[a]A, HO-MEM medium + 4% of fetal-calf serum; B, BHI + hemin + 3% of fetal-calf serum.

depends on the growth medium. Cells grown on medium A contained mainly mannose, with smaller proportions of ribose, galactose, glucose, and inositol. However, cells grown on medium B contained a much lower proportion of mannose, with an increase in the quantities of glucose and ribose, the latter arising from RNA components in the two cell preparations.

The ratio of the component sugars of cells grown on medium A, namely mannose, galactose, glucose, and inositol, resembled somewhat that of polysaccharide A (Table I), which suggests that most cell carbohydrates were present in polymeric forms. However, extraction of cells grown on medium B resulted in a drastic reduction of the proportion of mannose from 28 to 7%, and in the appearance of a significant proportion of arabinose (10%), Thus, these cells probably contain short chains of mannosyl residues, which are not isolated in the ethanol-precipitation step. This agrees with the isolation of a glycoprotein[9] whose component sugars consisted of 60% of mannose.

The difference in structure of polysaccharides A and B was also reflected in the H-1 regions of the ^{1}H-n.m.r. spectra (Fig. 1, spectra **1** and **2**, respectively).

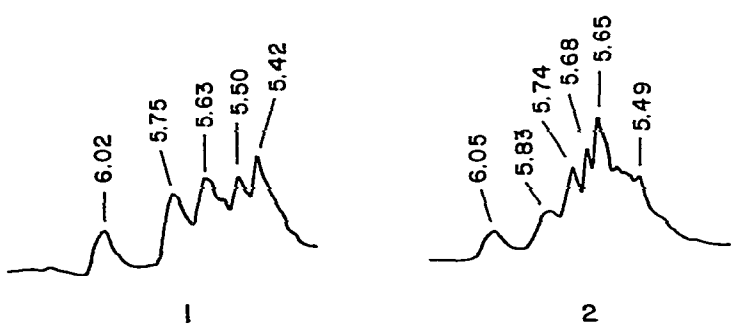

Fig. 1. H-1 region of ^{1}H-n.m.r. spectra of polysaccharides A (**1**) and B (**2**) in solutions in deuterium oxide at 70°. Numerical values are chemical shifts (δ) relative to external Me_4Si in a capillary.

TABLE II

O-METHYLALDITOL ACETATES FROM METHYLATED POLYSACCHARIDE OF *L. tarentolae*

Position of O-methyl group and parent sugar	*Proportion (% of peak area)*		*Comparative retention-time*[a]
	Polysaccharide of cells grown in Medium A	*Polysaccharide of cells grown in Medium B*	
2,3,5-Ara		8	0.62
2,3,4,6-Man	4	1	1.00
2,3,4,6-Glc	13	25	1.03
2,3-Ara		13	1.09
2,3,4,6-Gal		1	1.14
3,4,6-Man	20	9	1.64
2,3,6-Man	5	6	1.77
2,3,6-Gal	9		1.81
2,3,6-Glc	31	24	2.00
2,3,4-Gal		0.5	2.31
2,6-Gluc	7	8	2.64
3,6-Gal	3		2.88
2,3-Man	3		3.42
3,4-Man	5	3	3.46

[a]Retention time of the corresponding alditol acetates on the OV-225 capillary column, relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylmannitol. The program was from 95 to 230° (at 2°/min).

The methylation data (Table II), obtained for crude polysaccharides A and B are of immunological significance. Probably the most important structural features are the nonreducing end-group, mannopyranosyl (4%) and glucopyranosyl (13%) in polysaccharide A, and arabinofuranosyl (8%), mannopyranosyl (1%), and glucopyranosyl (25%) in polysaccharide B. In both polymers, the mannopyranosyl residues have the α-D configuration, as they are cleaved by α-D-mannosidase[10] to give D-mannose, detected on paper chromatograms. These nonreducing end-groups differ from those of the related protozoan, *Trypanosoma cruzi*, which are α-D-mannopyranosyl (26%), β-D-galactofuranosyl (9%), and galactopyranosyl (1%)[11]. Thus, any serological cross-reaction occurring between *L. tarentolae* and *T. cruzi* would be due to α-D-mannopyranosyl units in the polymeric form just described, or as components of short chains in glycoproteins.

Because of the difficulties in growing large quantities of cells of *L. tarentolae* and in the low yield of polysaccharide (1%), it was not possible to fractionate polysaccharides A and B in sufficient amounts to perform detailed structural investigations. However, even so, some conclusions could be reached, particularly with polysaccharide B, which could be produced in greater quantity because of better growth in medium B.

Perhaps the most interesting feature of polysaccharide B is the presence of arabinose. However, insufficient quantities were available to determine its configura-

tion. The methylation analysis provided 2,3,5-tri-*O*-methylarabinitol acetate (8%), corresponding to nonreducing end-groups of arabinofuranose. Also, the presence of 2,3-di-*O*-methylarabinitol acetate (13%) indicates either 4-*O*-substituted arabinopyranosyl or 5-*O*-substituted arabinofuranosyl residues (or both). The absence of derivatives of mono-*O*-methyl or unsubstituted arabinose shows that the arabinosyl residues are part of a heteropolymer. Previously, an arabinogalactan having a main chain of (1→3)-linked β-D-galactopyranosyl units, partially substituted at O-2 by D-arabinopyranosyl residues had been isolated from the protozoan *Crithidia fasciculata*[6].

Polysaccharide B contains a high proportion of glucose (70%) and has a specific rotation of +79°. Since this value is higher than that of +12° observed for polysaccharide A, which contains only 6% of glucose, it would appear that the D-glucopyranosyl residues have the α-D configuration. Color tests with iodine precluded the presence of amylose, amylopectin, or glycogen. However, D-glucose was formed by the concurrent action of α-amylase and amyloglucosidase[12], indicating the presence of a branched structure with 4-*O*- and 4,6-di-*O*-substituted α-D-glucopyranosyl residues.

Partial acetolysis[13] of polysaccharide B gave a mixture corresponding, on a paper chromatogram, to mannose, galactose, glucose, 2-*O*-α-D-mannopyranosyl-D-mannose, *O*-α-D-mannopyranosyl-(1→2)-*O*-α-D-mannopyranosyl-(1→2)-D-mannose (trace), and *myo*-inositol. The α-D configuration for the mannosyl units agrees with the cleavage (described earlier) of the polysaccharide by α-D-mannosidase, and the suggested (1→2) linkages are consistent with the methylation data.

EXPERIMENTAL

Culture conditions. — *Leishmania tarentolae*, strain TAR II, UCI variant, was kindly supplied by Professor Fernando S. da Cruz, Universidade Federal do Rio de Janeiro, Brazil. Cells were grown on two different media, one a monophasic medium (designated HO-MEM) is a modified Eagle's minimal, essential medium with Spinner's salts and 4% of fetal calf serum, and utilizes D-glucose as carbon source; it is referred to as medium A[7]. The other medium (designated B) is complex and is made of brain heart infusion (BHI) containing 25 μg of hemin/mL and 3% of fetal-calf serum[8]. Cells were grown in stationary culture in 500-mL Erlenmeyer flasks for 7 days at 28°.

Isolation of glycoprotein. — Glycoprotein was isolated in crude form by extraction with aqueous phenol[9].

Isolation of polysaccharides. — Cells were isolated by centrifugation and washed three times with physiological saline. They were extracted with 6% aqueous potassium hydroxide (200 mL) for 6 h at 100°, and the solution was neutralized with acetic acid and centrifuged. The supernatant was concentrated to a small volume and added to ethanol (3 vol.). The resulting precipitate was isolated by filtration, dissolved in water, and the solution passed through a mixed-bed resin column to remove nucleic acids.

The eluate was concentrated to a small volume, and the polysaccharide reprecipitated with ethanol and isolated.

The yields of dry cells per liter of medium, and percentage yields of polysaccharide, based on cell weight, were as follows: HO-MEM medium (medium A), 1.5 g, 1%; and complex medium (medium B), 1.5 g, 1.5%.

Detection and analysis of component sugars of polysaccharides. — Monosaccharides arising from cells or potassium hydroxide extracts were detected[14] by paper chromatography, following hydrolysis with 0.5M sulfuric acid for 18 h at 100° (solvent: 40:11:19, v/v, 1-butanol–ethanol–water; spray: *p*-anisidine hydrochloride). Quantitative estimations were performed by reduction of the resulting sugars with sodium borohydride, followed by acetylation and g.l.c. of the alditol acetates[15].

Methylation of polysaccharides. — The procedures of Haworth[16] and Kuhn[17] were used successively, and the resulting per-*O*-methylated product was degraded to a mixture of *O*-methylalditol acetates, which were identified by g.l.c.–m.s.[18], on two different, 30-mm glass-capillary columns. The liquid phases were OV-225 and OV-17, programmed from 95 to 230° (at 2°/min). OV-225 proved satisfactory for qualitative and quantitative purposes (see Table II).

Partial acetolysis. — The procedure of Lee and Ballou was used[13]. Sugars obtained on *O*-deacetylation were examined on paper chromatograms (solvent: 2:1:1, v/v, 1-butanol–ethanol–water; sprays: *p*-anisidine hydrochloride and ammoniacal silver nitrate).

Enzymic degradation. — Samples (3 mg) of polysaccharides obtained from both media were treated with exo-α-D-mannosidase[10] (5 mg) in water (0.2 mL). The reaction was terminated after 8 h, by heating, the solution was de-ionized, and the product examined on a paper chromatogram.

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