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Cell Walls of *Coccidioides immitis*: Neutral Sugars of Aqueous Alkaline Extract Polymers

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The neutral sugar components of hydrolysates of the alkali-soluble and -insoluble fractions of an acid-extracted *Coccidioides immitis* mycelial cell wall preparation were examined by gas-liquid chromatography. The alkali-soluble fraction was separated into a neutral water-soluble (46% carbohydrate) and a neutral water-insoluble fraction (6% carbohydrate). Glucose was the major neutral sugar constituent of all fractions. Mannose appeared to be a second major component of the alkali-soluble, water-soluble fraction. 3-*O*-methylmannose was absent. Small amounts of other sugars, including galactose and pentoses, were tentatively identified. Borohydride reduction before alkaline extraction resulted in retention of almost half of the glucose and variable amounts of other components in the alkaline residue, indicating that solubilization of cell wall polymers by alkaline extraction results in significant degradation of the native cell wall polymers.

As part of an ongoing program aimed at attempting the isolation and characterization of coccidioidin components at the molecular level, we have been systematically studying various fractions of culture filtrates, cell walls, and autolysates of *Coccidioides immitis*. Previous work indicated the presence of 3-*O*-methylmannose and mannose as the main components, in addition to other constituents in serologically active and skin test-active fractions (1, 3). Also we observed previously that the mycelial cell wall of *C. immitis* contained both acid- and alkali-extractable nitrogen-containing polymers (8; E. Scher and R. Wheat, Bacteriol. Proc., p. 118, 1971). We now report the gas-liquid chromatographic identification of neutral sugars found in the alkali-soluble and -insoluble fractions of *C. immitis* mycelial cell walls. Since completion of the present work, a report appeared indicating that alkali-soluble, water-soluble extracts of *C. immitis* mycelial cell walls exhibit delayed hypersensitin activity (7). That report and the present results raise a question as to whether 3-*O*-methylmannose and mannose are part of the delayed hypersensitin component of coccidioidin.

Mycelia of *C. immitis* Silveira, grown as described previously (1), were killed in acetone, defatted further by chloroform-methanol (2:1) extraction (three times) in a Waring blender, extracted (twice) with aqueous 45% phenol at 70°C to remove soluble polysaccharides, proteins, and lipids, and dialyzed. The residue was extracted with 1 M perchloric acid at 90°C for 15 min to remove deoxyribonucleic acid. The residue was washed with water, extracted once more with aqueous phenol followed by chloroform-methanol (2:1) extraction, and then acetone dried. The yield of cell walls prepared by this procedure was 24%.

Alkaline extraction of this mycelial cell wall preparation by three sequential extractions with 1 M NaOH at room temperature, by the procedure of Kanetsuna and Carbonell (2), solubilized ca. 30% of the dry weight, leaving the bulk of the wall material in the alkali-insoluble fraction. When the alkali-soluble extract was neutralized with acetic acid, a precipitate formed, yielding, after centrifugation, dialysis, and freeze-drying, insoluble and soluble components in almost equal amounts (i.e., 2 to 3% each of the mycelial wall). (Since only 70 to 75% recovery of the cell wall material was achieved, the alkali treatment apparently also produced soluble dialyzable or volatile products.) Gas-liquid chromatographic analysis of these fractions by previously described procedures (1, 4-6) revealed the neutral sugar components shown in Table 1. Of interest is the immediately obvious fact that the cell wall preparation is devoid of the 3-*O*-methylmannose-, mannose, and galactose-containing polymers found in the immunologically active culture filtrates, autolysates, and aqueous phenol and perchloric acid extracts studied previously (1, 4, 6). Both of the alkali-soluble mycelial wall fractions contained

TABLE 1. *Carbohydrate content of acid-insoluble mycelial cell wall and alkaline extract fractions of C. immitis*

Sugar component[a]	Cell wall	Alkali-soluble after neutralization		Alkali-insoluble borohydride treatment	
		Water soluble	Water inolu-ble	+	–
Glucose	1,930	1,904	166	949	81
Mannose	105	435	31	87	
(Galactose)	Tr[b]	72			12
(Xylose)	97	74	55	6	
(Ribose)	Tr	4	4	4	
(Arabinose)	Tr	45	60	10	
(Erythrose)		17	40	5	
Others		26	22		
Percent yield	100	1.7	2.3	66	68.6
Percent as glucose	38.2	46	6	17.6	1.7

[a] Parentheses indicate tentatively identified components.

[b] Tr, Trace.

glucose and mannose as major constituents, although the neutral, aqueous soluble fraction contained some 10-fold or more of the amounts found in the neutral, aqueous insoluble fraction. In addition, small amounts of a compound tentatively identified as galactose were found only in the water-soluble fraction of the aqueous alkaline extract. Somewhat unexpected (cf. 1, 8) was the presence, in both alkaline extract fractions, of small amounts of compounds tentatively identified as pentoses, erythrose and even smaller amounts of deoxyribose, fucose, and rhamnose (the latter three are designated as "others" in Table 1). The presence of a trace of 2-deoxyribose in only the alkali-soluble, water-soluble fraction may indicate a residuum of deoxyribonucleic acid that was not removed by the perchloric acid treatment; it is also possible that the "deoxyribose" may be an alkaline degradation product of some other cell wall component. Because of the possibility that the small amounts of "pentoses" and other previously non-reported sugar components observed in the alkaline extract fraction could be oxidative alkaline degradation products, their identification is considered tentative. To test the possibility of alkaline degradation, a portion of the cell wall preparation was treated with an equal weight of sodium borohydride at pH 7.2 before alkaline extraction in parallel with the same amount of non-borohydride-treated cell wall preparation. Both alkaline residue fractions were then examined for neutral sugars. The results (Table 1) show that a significant amount of glucose and mannose, in addition to the compounds tentatively identified, were retained in the borohydride-reduced alkaline residue fraction, indicating that degradative solubilization of cell wall components occurs in alkali. It is obvious that much remains to be done to define and correlate at the molecular level the composition, structure, and interrelationship of the chemical constituents and the biologically active components of the cell wall of *C. immitis*.

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