Synthesis of Monorhamnosyl L-Rhamno-D-Mannans by Conidia of Sporothrix schenckii

L. R. TRAVASSOS†* AND L. MENDONÇA-PREVIATO

Departamento de Microbiologia Geral, Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 20000, Brazil

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A rhamnomannan containing single-unit α-L-rhamnopranosyl side chains was identified in isolated conidia from Sporothrix schenckii. Such a rhamnomannan differed from the dirhamnosyl rhamnomannan synthesized by the hyphae but was very similar to the monorhamnosyl rhamnomannan formed in yeastlike cells. Nuclear magnetic resonance spectroscopy and chemical analysis were used to compare these polysaccharides. Based on the distribution of different rhamnomannans in different S. schenckii cell types and in view of the reactivity of some human antisera previously reported (6), the formation of hyphae in vivo is suggested.

Previous studies (8, 13) have shown that the yeast forms of the human pathogen Sporothrix schenckii synthesize rhamnomannans in which a (1 → 6)-linked α-D-mannopyranose main chain is substituted in the 3-O positions by α-L-rhamnopranose nonreducing end units. This monorhamnosyl rhamnomannan differed from the mycelial dirhamnosyl rhamnomannan, in which the side chains consisted mainly of O-α-L-rhamnopranosyl-(1 → 2)-α-L-rhamnopranose residues. Another structural feature of S. schenckii rhamnomannans was the presence of 4-O- and 2,4-di-O-substituted α-D-mannopyranose units which occur in both yeast and mycelial polysaccharides (4, 8, 13).

Mycelial cultures of S. schenckii were usually obtained at 25°C. With various growth media, particularly the semisynthetic ones, these cultures consisted of hyphae and conidia, whereas yeastlike cells only formed when the incubation temperature was raised to 35 to 37°C. Since in our previous communications (8, 13) no attempt was made to separate hyphae and conidia, a direct correlation between cell type and the synthesis of a particular polysaccharide was not possible in this case. In one instance, however, the unsporulated mycelium culture of one strain of S. schenckii (1099.18) was obtained and shown to synthesize a typical dirhamnosyl rhamnomannan (8). We now extend this study by determining the nature of the conidial antigen.

The recognition of different antigens in different cell types of S. schenckii associated with previous data on the immunological response of human patients with sporotrichosis (6) suggests that hyphae are formed in vivo.

MATERIALS AND METHODS

Microorganism. S. schenckii strain 1099.18 was isolated from a human case of sporotrichosis. It was obtained from the Mycology Section of the Department of Dermatology, Columbia University, New York. Stock cultures were maintained at 4°C in solid Sabouraud medium distributed in tubes with a layer of mineral oil.

Isolation of conidia. S. schenckii (1099.18) was grown in the YCV (yeast nitrogen base [Difco], Casamino Acids [BBL], and vitamins) medium at 25°C for 7 days as before (13). The culture contained hyphae and conidia but no yeastlike forms. It was filtered twice through gauze to separate conidia from hyphae. The filtrate was an almost pure suspension of conidia.

Isolation and analysis of the conidial rhamnomannan. Conidia were extracted with hot dilute KOH (2%), and the rhamnomannan was purified by precipitation with Fehling reagent as described before (8, 13). Total carbohydrate, nitrogen, and phosphate were determined by the phenol-sulfuric acid method (2), the ninhydrin method (11), and the method of Ames (1), respectively. Quantitative determination of monosaccharides in the polysaccharide preparation was performed by gas-liquid chromatography of the corresponding alditol acetates (8, 10). A column (6 feet by 0.125 inch [ca 182.9 by 0.32 cm] in diameter) with neopentylglycol-succinate (3%) in 100-120 Gas Chrom Q at 210°C was used. Methylation of the polysaccharide and identification of the partially methylated alditol acetates were carried out as described before (13).

NMR spectroscopy. Proton and 13C nuclear magnetic resonance (NMR) spectra of the conidial polysaccharide were obtained by using a Varian XL-100 spectrometer from 100-mg/ml solutions in D2O at 70°C with tetramethylsilane as the external standard. A detailed description of the procedure is given elsewhere (8, 14).

† Present address: Memorial Sloan-Kettering Cancer Center, New York, NY 10021.
RESULTS

Conidia of S. schenckii (1099.18) formed a rhamnomannan and a galactose-containing polysaccharide, probably a galactan as in Cercospora stenoceras (4). Although purification via precipitation with Fehling reagent enriched the rhamnomannan fraction, this procedure did not completely eliminate the galactan component, part of which (6.2%) co-precipitated with the rhamnomannan. This minor component did not interfere with the analysis of the rhamnomannan. Analytical data on the conidial polysaccharide are given in Table 1. The proportions of rhamnose and mannose in the polysaccharide agree with those generally found for monorhamnosyl rhamnomannans. By use of the Hakomori procedure (8,13) for methylation of the polysaccharide, it was observed that one single methylation was sufficient for the rhamnomannan, whereas the galactan remained largely unmethylated. Thus the partially methylated derivatives that arose in the methylation analysis of the rhamnomannan were not mixed with the correspondent derivatives of the galactan. The methylated derivatives from the rhamnomannan methylation analysis were: 2,3,4-trimethyl-1,5-diacyethyl-rhamnitol (1.0); 3,4, dimethyl-1,2,5-triacyethyl-rhamnitol (0.08); 3,4,6-trimethyl-1,2,5-triacyethyl-mannitol (0.07); 2,3,6-trimethyl-1,4,5-triacyethyl-mannitol (0.27); 2,3,4-trimethyl-1,5,6-triacyethyl-mannitol (0.05); and 3,6-dimethyl-1,2,4,5-tetra-acetyl-mannitol and 2,4-dimethyl-1,3,5,6-tetra-acetyl-mannitol. The relative proportions of the dimethyl derivatives of mannitol were not calculated due to their extensive degradation. The proportion of the 3,4-dimethyl derivative of rhamnitol, less than 10% of that of the 2,3,4-trimethyl derivative, suggests that only a very small percentage of the side chains contain (1→2)-linked di-rhamnosyl residues. The 13C NMR spectrum of the polysaccharide as described below also shows that 2-O-substituted a-L-rhamnopyranose units are present in minor concentrations in this polysaccharide (Fig. 1).

The proton NMR spectrum (H-1 region) of the conidial polysaccharide (Fig. 2) was very similar to those of S. schenckii rhamnomannans obtained at 37°C or those of C. stenoceras polysaccharides (14). Signals at δ 4.12, 4.25, and 4.51 were present, but a signal at δ 4.40 to 4.42 was absent. The latter is consistently present in spectra of S. schenckii di-rhamnosyl rhamnomannans (13,14). The 13C NMR spectrum of the conidial rhamnomannan (Fig. 1) showed peaks at δ 102.3, 101.0, 100.1, and 98.2 in the C1 region, which is characteristic of spectra of monorhamnosyl rhamnomannans. Individual assignments of the C1 signals were made previously (4, 8, 14). A signal at δ 18.4 corresponds to the methyl group of the rhamnopyranose units, thus being characteristic of all rhamnomannans. Only traces of 2-O-substituted α-L-rhamnopyranose units were suggested by the presence of very minor peaks at δ 103.5 and 80.2 (C1 of the α-L-Rhap nonreducing end unit of α-L-Rhap-(1→2)-α-L-Rhap and C2 of α-L-Rhap 2-O-substituted, respectively (8)), although the peak at δ 96.7 (C1 of α-L-Rhap 2-O-substituted [14]) was not evident in the spectrum of the conidial polysaccharide. The signals at the δ 77.0 to 66.7 region, with the exception of that at δ 75.8, are the same (corrected for 70°C) as those observed previously in the spectrum of the monorhamnosyl rhamnomannan from S. schenckii (1099.18) yeastlike forms (4).

DISCUSSION

S. schenckii mycelial cultures synthesize a mixture of polysaccharides consisting mainly of different types of rhamnomannans. The unsporulated mycelium culture of S. schenckii strain 1099.18, obtained in a semisynthetic medium (12) with short incubation, formed a di-rhamnosyl rhamnomannan (8).

In another S. schenckii strain (1099.12) minor concentrations of a galactomannan were isolated from a conidia-less mycelium culture at 25°C. The homogeneity of this strain is, however, uncertain. Originally described as a pathogenic mutant of C. stenoceras (7), it was reclassified as S. schenckii (9) on the basis of deoxyribonucleic acid hybridization studies. In a semisynthetic medium permitting growth of S. schenckii (1099.12) with mixed morphology, a typical di-rhamnosyl rhamnomannan was isolated at 25°C (13). When a yeast culture of this strain was plated on Sabouraud agar at 25°C, at least two different mycelial populations emerged in subcultures from different clones. These were recognized on the basis of their different rhamnomannans (Travassos et al., submitted for publication). Under the conditions used for the isolation of the galactomannan from the unspor-
lated mycelium of strain 1099.12, a rhamnose-containing polysaccharide was not detected simultaneously (8). Furthermore, a similar galactomannan has not so far been recognized in other S. schenckii strains. The instability or heterogeneity of S. schenckii strain 1099.12 suggested by these experiments prompted us to concentrate our efforts on characterizing the surface rhamnomannans from a typical S. schenckii strain (1099.18) originally isolated from a human case of sporotrichosis.

In studies carried out with S. schenckii grown at 25°C, cultures usually consisted of either hyphae and conidia or unsporulated mycelia. No direct analysis of the polysaccharide from isolated conidia has been reported before. Based on chemical data and NMR spectroscopy, we now show that the conidial rhamnomannan is very similar to that formed by S. schenckii yeastlike cells, thus differing from the dirhamnosyl rhamnomannan synthesized by the hyphae. Both the methylation data and the 13C NMR spectrum indicated the presence of a very minor proportion of 2-O-substituted α-L-rhamnopyranose units and thus of (1 → 2)-linked di-rhamnosyl side chains in the polysaccharide. The conidial rhamnomannan consists of a (1 → 6)-linked α-D-mannopyranose main chain substituted in positions 3-O by α-L-rhamnopyranose nonreducing end units. This polysaccharide also contains a few 4-O- and 2,4-di-O-substituted α-D-mannopyranose units, which are characteristic of S. schenckii rhamnomannans synthesized at 25°C (8).

Previous results (6) have demonstrated that some human antisera from patients with sporotrichosis reacted more strongly with the dirhamnosyl rhamnomannans, whereas others reacted equally well with mono- or dirhamnosyl rhamnomannans. These studies also showed that a rabbit antiserum reacting mainly with the dirhamnosyl residues of the rhamnomannan was more specific than another serum raised against cells synthesizing predominantly monorhamnosyl rhamnomannans. The stronger reactivity of human antisera against the dirhamnosyl rhamnomannans can only be explained on the basis of the intermediate formation of hyphae in the infected tissue. In fact, yeastlike forms synthesize monorhamnosyl rhamnomannans, and present data show that conidia also synthesize a
polysaccharide, mostly with single-unit \(\alpha\)-rhamnose side chains. Apparently, conidia do not give rise directly to yeast forms (3); thus the intermediate formation of hyphae seems to be an obligatory step in vivo. This assumption is probably true for \(S.\) schenckii strain 1099.18, in which the antigens from all cell types have been studied in detail. It is likely that other strains behave similarly, since we have shown that dirhamnosyl rhamnomannans are usually synthesized by \(S.\) schenckii mycelial cultures rather than by yeastlike forms (13, 14). Hyphae in the human tissue of sporotrichosis have already been demonstrated by Lii and Shigemi (5).

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LITERATURE CITED