Binding Characteristics of Lectins Involved in the Trapping of Nematodes by Fungi

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Seventeen saccharides were tested for their ability to bind to the trap lectins of three species of nematode-trapping fungi and prevent nematode capture. The lectin of Arthrobotrys conoides was found to be inhibited by α-D-glucose/b-mannose and similar saccharides. The lectins of Monacrosporum eudermatum and Monacrosporum rutgeriensis were inhibited by α-L-fucose and 2-deoxy-D-glucose, respectively. Human group O(H) erythrocytes agglutinated to traps of M. eudermatum but not A. conoides or M. rutgeriensis. There was no agglutination of group A or B to traps formed by all three fungi. Exposure of the traps to trypsin eliminated the ability to capture nematodes. The presence of D-glucose/b-mannose and L-fucose residues on the nematode cuticle was suggested through the use of commercially prepared lectin-peroxidase conjugates.

Nematode-trapping fungi are a group of predatory microcarnivores that can capture living nematodes, which they kill and digest (14). Capture of the nematode is achieved either by adhesion (1, 4, 5, 13) or through the use of nonadhesive rings (5, 15, 19). Once captured, the nematode dies and its cuticle is penetrated, probably through the use of a collagenase (20). Fungal hyphae then grow throughout the dead nematode, it is enzymatically degraded, and the soluble nutrients are assimilated by the fungus (13, 14).

Recent evidence (10-12) indicates that for two fungal species, Arthrobotrys oligospora and Dactylaria candida, the initial event in the capture of the nematode is mediated by a lectin present on the trap and specific saccharides on the nematode cuticle (N-acetylgalactosamine for A. oligospora and 2-deoxyglucose for D. candida).

The present investigation was conducted to determine the binding specificities of the trap lectins produced by Arthrobotrys conoides, Monacrosporum eudermatum, and Monacrosporum rutgeriensis.

MATERIALS AND METHODS

Source and maintenance of nematode-trapping fungi. A. conoides, M. eudermatum, and M. rutgeriensis were obtained from the collection of cultures maintained in this laboratory. The fungi were maintained on cornmeal agar (Difco Laboratories).

Maintenance and cultivation of nematodes. The nematode Panagrellus redivivus was maintained and cultured for studies, axenically, by the procedures of Rothstein (17, 18).

Determination of the saccharide-binding characteristics of the trap lectin. Binding characteristics of the trap lectin were determined by the procedure of Nordbring-Hertz and Mattiasson (9, 12). The fungi were grown for 72 to 96 h at 21°C on a strip (20 by 70 mm) of dialysis membrane (Technilab 299; molecular weight cutoff, 6,000; A. H. Thomas Co.) placed on the surface of a petri plate of the low nutrient medium of Nordbring-Hertz and Mattiasson. After 72 to 96 h, 1 drop of a washed nematode suspension (30 to 50 nematodes in phosphate-buffered saline [PBS], pH 6.8) was added to each fungal colony to induce trap formation, and the plates were incubated for an additional 48 h. After 48 h, the dialysis membrane strips and attached fungal growth were transferred to an empty petri plate (to prevent further growth and trap formation). The fungal colony was then flooded with a solution of the test saccharide and incubated for 24 h. A 20, 200, and 400 mM solution of each saccharide in PBS (pH 6.8) was tested. High and low concentrations of the saccharide were used to determine whether binding specificity is influenced by saccharide concentration, as has been reported elsewhere (6, 12). Saccharides tested included D-glucose, 2-deoxy-D-glucose, D-mannose, α-methyl-D-mannoside, D-fructose, N-acetyl-D-galactosamine, D-galactose, 2-deoxy-D-galactose, L-fucose, N-acetyl-D-galactosamine, L-sorbose, L-xylulose, D-arabinose, D-melibiose, maltose, sucrose, and lactose (Sigma Chemical Co.). Control solutions consisted of PBS. After 24 h, the excess saccharide was removed from the colony, a few drops of a washed nematode suspension was added, and the colony was examined microscopically (×100) over a 6-h time period for nematode capture. Differentiation between nematodes captured during the trap induction step and this step can be easily determined by the amount of decomposition the nematode has undergone. Nematodes trapped during the induction step are greatly decomposed in comparison to those cap-
TABLE 1. Effect of various saccharides on the ability of A. conoides to trap nematodes

<table>
<thead>
<tr>
<th>Saccharide</th>
<th>Trapping ability* at:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>20 mM</td>
</tr>
<tr>
<td>N-Acetylgalactosamine</td>
<td>ND*</td>
</tr>
<tr>
<td>N-Acetylgulcosamine</td>
<td>ND*</td>
</tr>
<tr>
<td>Glucose</td>
<td>–</td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td>–</td>
</tr>
<tr>
<td>Mannose</td>
<td>–</td>
</tr>
<tr>
<td>α-Methylmannoside</td>
<td>–</td>
</tr>
<tr>
<td>Fructose</td>
<td>ND</td>
</tr>
<tr>
<td>Arabinose</td>
<td>–</td>
</tr>
<tr>
<td>Melibiose</td>
<td>ND</td>
</tr>
<tr>
<td>Maltose</td>
<td>–</td>
</tr>
</tbody>
</table>

* Saccharides having no influence on trapping at all concentrations were galactose, 2-deoxygalactose, fructose, sorbose, xylose, lactose, and sucrose.

To determine the presence of glucose/mannose residues on the cuticle, 100 μg of concanavalin A (ConA; Sigma Chemical Co.), a glucose/mannose-binding lectin, was added to 1 ml of a washed nematode suspension (30 to 50 nematodes per ml) and incubated at 21°C for 30 min. After six washes with PBS to remove unadsorbed ConA, 200 μg of horseradish peroxidase (Sigma Chemical Co.) was added to 1 ml of the nematode suspension and incubated an additional 30 min at 21°C. The nematodes were then washed six times in PBS to remove unbound peroxidase and resuspended in 0.6 ml of PBS. The nematode suspension was then assayed spectrophotometrically (505 nm; Beckman model 24 spectrophotometer) for peroxidase activity by the procedure of Lott and Turner (8), using 0.6 ml of a substrate consisting of 28 mM phenol, 1.6 mM 4-aminoantipyrine, and 4 mM hydrogen peroxide and an assay time of 10 min. The spectrophotometer was first zeroed in with a blank consisting of 0.6 ml of substrate and 0.6 ml of PBS. Controls consisted of nematode suspensions exposed to either ConA or peroxidase only.

To determine the presence of L-fucose residues on the nematode cuticle, 1 ml of a washed nematode suspension was incubated with 250 μg of an L-fucose-specific lectin-peroxidase conjugate (Lotus tetragonolobus lectin-horseradish peroxidase; E-Y Laboratories, Inc.) at 21°C for 30 min. The nematodes were then washed six times in PBS to remove unadsorbed lectin-peroxidase conjugate, resuspended in 0.6 ml of PBS, and assayed for peroxidase activity as described above.

RESULTS AND DISCUSSION

A. conoides, M. eudermatum, and M. ruteriensi appear to produce a trap lectin that plays a role in nematode capture. Seventeen saccharides were tested for their ability to bind to the trap lectins and prevent capture of nematodes by the fungi. In general, the specificity of the trap lectins decreased as the saccharide concentration increased. At both 20 and 200 mM concentrations, the trapping ability of A. conoides was inhibited by α-D-glucose, 2-deoxy-D-glucose, D-mannose, α-methyl-D-mannoside, D-arabinose, and maltose (Table 1). When the saccharide concentration was increased to 400 mM, trapping was prevented by N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, D-fructose, and D-melibiose in addition to the six previously mentioned saccharides.

M. eudermatum was only inhibited from trapping nematodes by α-L-fucose at all three concentrations tested (Table 2). D-Arabinose was inhibitory at 200 and 400 mM concentrations, whereas L-sorbose was inhibitory only at 400 mM.
At all three concentrations tested, 2-deoxy-D-glucose prevented nematode capture by M. rutgeriensis, whereas N-acetyl-D-glucosamine was inhibitory at the high concentration of 400 mM (Table 3). It has been suggested that for another nematode-trapping fungus (D. candida), the lectin also appears to be inhibited by 2-deoxy-D-glucose (11).

The loss of specificity observed here as the saccharide concentration increased has been reported for lectins of other nematode-trapping fungi (12) and of other organisms (6, 7, 16). The reason for this loss of specificity is still not clear.

When exposed to human A, B, and O(H) RBCs, only group O(H) agglutinated to traps of M. eudermatum. There was no agglutination of either A or B to M. eudermatum or any of the RBCs to A. conoides or M. rutgeriensis. The O(H) RBCs agglutinated only to the traps, and there was no attachment to hyphal filaments or spores.

RBCs carry different saccharides on their surfaces (22). These saccharides give the RBCs their specificity during blood grouping. The terminal saccharide of group A is N-acetyl-D-galactosamine, group B has D-galactose, and O(H) has L-fucose. Based on these terminal saccharides, it is not surprising that only O(H) RBCs agglutinated to traps of M. eudermatum. Results of the influence of saccharides on trapping suggested that the lectin of M. eudermatum was inhibited by L-fucose. The blood cell study tends to confirm this L-fucose specificity. A. conoides, being inhibited by D-glucose/D-mannose and similar saccharides, and M. rutgeriensis, being inhibited by 2-deoxy-D-glucose, would not be expected to agglutinate the RBCs tested.

Exposing the traps to trypsin totally eliminated trapping abilities of all three fungi, suggesting that the lectin material is proteinaceous in nature. Light microscopic examination of the trypsin-treated colonies revealed no apparent disruption of the fungal filaments or traps. In addition, when transferred to cornmeal agar, the trypsin-treated colonies had a growth rate that was not significantly different from that of non-trypsin-treated controls, indicating that the trypsin treatment did not affect fungal viability. A loss of trapping ability was also observed in A. oligospora and D. candida when the traps were treated with trypsin (11). Both these organisms produce lectin-containing traps (10-12).

Once the saccharide-binding characteristics of the trap lectins were determined, it was necessary to demonstrate the appropriate saccharides on the nematode cuticle. The presence of D-glucose/D-mannose residues (inhibitory of the lectin of A. conoides) were suggested by exposing the nematodes first to ConA and then to horseradish peroxidase. ConA has four binding sites (2) and can attach to the nematode cuticle and still have free binding sites to bind the peroxidase, which contains D-mannose residues as part of its structure (A. E. Chu, E. Y. Laboratories, personal communication). The peroxidase activity of the nematodes exposed to ConA and peroxidase (0.59 ± 0.149) was 12 times greater than that of the nematodes exposed to peroxidase alone (0.05 ± 0.011). Nematodes exposed to ConA alone had no peroxidase activity.

To demonstrate L-fucose residues (specific for the lectin of M. eudermatum) on the nematode cuticle, nematodes were exposed to an L-fucose-specific lectin-horseradish peroxidase conjugate and assayed for peroxidase activity. The peroxidase activity of the nematodes exposed to the lectin-peroxidase conjugate (2.40 ± 0.149) was 11 times greater than the nematodes exposed to peroxidase alone (0.22 ± 0.024). There was no peroxidase activity by the nematodes exposed to lectin alone.

A commercially prepared 2-deoxy-D-glucose-specific lectin-peroxidase conjugate is unavailable. Lectins that appear to bind 2-deoxy-D-glucose have only been described in one other

### TABLE 3. Effect of various saccharides on the ability of M. rutgeriensis to trap nematodes

<table>
<thead>
<tr>
<th>Saccharide</th>
<th>Trapping ability* at:</th>
<th>20 mM</th>
<th>200 mM</th>
<th>400 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetylglucosamine</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

- *Saccharides having no influence on trapping at all concentrations were N-acetylglucosamine, galactose, 2-deoxygalactose, N-acetylgalactosamine, glucose, 2-deoxyglucose, mannose, α-methylmannoside, fructose, xylose, melibiose, lactose, and sucrose.
- ND, Not determined.
- +, Normal capture of nematodes; -, inhibition of nematode capture.
study (11) dealing with nematode-trapping fungi and possibly in the white clover plant (3). Identification of 2-deoxy-D-glucose on the nematode cuticle was not undertaken.

In conclusion, the data presented here suggest that A. conoides, M. eudermatum, and M. rutgeriensis capture nematodes through the use of lectin-containing traps that are inhibited by various saccharides (α-D-glucose/β-mannose, α-L-fucose, and 2-deoxy-D-glucose, respectively).

ACKNOWLEDGMENTS

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LITERATURE CITED