Host-microorganism Interaction; Studies on the Molecular Mechanisms Behind the Capture of Nematodes by Nematophagous Fungi *

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The understanding of the biological significance behind receptor–ligand interactions as a basis for cell–cell interactions is rapidly increasing.[1] Developmentally regulated lectins have been identified in cellular slime molds, and lectin activity is closely correlated with the development of cohesiveness between the cells. The role of lectins in the *Rhizobium* – leguminous plant microorganism–host interrelationship is well documented.[2] Furthermore, fungal lectins are produced in the fungi–algae symbiotic relationship in lichens.[3]

Recently, evidence was presented demonstrating that a developmentally regulated lectin is present on the capture organs of *Arthrobotrys oligospora* Fres. and is responsible for binding to surface structures of the nematode (*Panagrellus redivivus*, Goodey) cuticle.[4] The nematode trapping ability was specifically inhibited by *N*-acetyl-D-galactosamine even if other galactose derivatives at increased concentrations could prevent or at least delay capture. This report deals with further studies on the trap lectin.

Experimental. A. oligospora was grown on dialysis membranes placed on agar-surfaces, and trap formation was initiated as described elsewhere.[5] To study the binding properties of the proposed lectin, trap-containing mycelium was washed from the dialysis membranes with distilled water and the cell material was collected in a test tube. The fungus was washed twice using centrifugation and decantation methods with buffer (0.1 M triethanolamine-HCl, pH 7.0, containing 1 mM each of Ca(II), Mg(II), Mn(II), Zn(II), K(I) and Na(I)). These metal ions are the most frequent in other metal dependent lectins. This buffer was used throughout. The fungus material was then suspended in buffer containing *N*-acetyl-D-galactosamine (100 mM) to protect binding sites of the trap lectin. The intact fungal material (*ca.* 8 mg dry weight) was then treated with 4-sulfo-2-[^{125}I]iodobenzenediazonium chloride (7.4 MB_q) (New England Nuclear, Boston, Mass. U.S.A.) in the buffer for 2 h at room temperature, before it was repeatedly washed with buffer to eliminate excess 4-sulfo-2-[^{125}I]iodobenzenediazonium chloride. The iodinated fungal material was homogenized in a Potter-Elveheim homogenizer containing fresh buffer plus protease inhibitor – 2-methyl-benzenesulfonylfluoride (0.02 mM). The mixture was cooled in ice prior to and during homogenization. The homogenate was dialyzed overnight at 4 °C against 10 mM triethanolamine –HCl buffer pH 7.0 being 1 mM with regard to Ca(II), Mg(II), Mn(II), Zn(II), K(I) and Na(I). This buffer was changed three times.

The binding of this preparation to both an *N*-acetyl-D-galactosamine-substituted Sepharose 6B [6] and to nematodes was investigated. A typical binding experiment was carried out according to the following procedure: *N*-Acetyl-D-galactosamine-Sepharose (100 mg well sucked), 200 µl buffer (100 mM triethanolamine-HCl containing the ions as described earlier) and 400 µl homogenate were mixed in a test tube. Incubation lasted for 2 h and was followed by repeated washing (5 times) with buffer of the Sepharose-preparation prior to counting in an LKB-Wallac 1275 Minigamma.

Results. Since the degree of development of capture organs on the fungus as well as the degree of incorporation of ^{125}I varied from experiment to experiment the degree of binding is presented as per cent. The above described experiment was set to equal 100 % (counts normally 8000–12 000 CPM). When the incubation was performed in the presence of 0.1 M *N*-acetyl-D-galactosamine, binding to the matrix was reduced to 30–32 %. These results verified the observations reported earlier on living organisms.[4] Since Sepharose *per se* contains structures that might interfere with the lectin, a blank experiment using hydrolyzed epoxi-Sepharose was performed. Here 24 % of the counts were bound in the absence of *N*-acetyl-D-galactosamine and 15 % in its presence. To verify that the trap receptor is a protein, specified portions of the homogenate were incubated with trypsin (10 mg trypsin/ml homogenate; incubation for 1 h at 37 °C). As a blank a sample without trypsin was used. As is seen from Table 1 only 15 % of ^{125}I was recovered from the gel, *i.e.*, the same amount as the background obtained when using hydrolyzed epoxi-Sepharose. From these results it can be deduced that the receptor molecule on the

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Table 1. Concentration of ^{125}I-labelled homogenized capture organs bound to *N*-acetyl-D-galactosamine Sepharose (mean of 2–5 experiments). Experimental conditions are given in the text.

Substance	%
N-acetyl-D-galactosamine Sepharose	
Homogenate	100
Homogenate + *N*-acetyl-D-galactosamine (100 mM)	30
Trypsin-treated homogenate	15
Hydrolyzed epoxi-Sepharose	
Homogenate	24
Homogenate + *N*-acetyl-D-galactosamine (100 mM)	15

Table 2. Concentration of ^{125}I-labelled homogenized capture organs bound to washed nematodes (mean of 2–5 experiments). Experimental conditions are given in the text.

Substance	%
Homogenate	100
Homogenate + *N*-acetyl-D-galactosamine (100 mM)	20–50
Homogenate + trypsin-treated nematodes	67

capture organs with specificity for *N*-acetyl-D-galactosamine is of protein nature. Furthermore, it seems likely that the background adsorption of ^{125}I is nonspecific since even after trypsin treatment 15 % remains bound.

Characterization of the nematode surface structures binding with homogenate was also performed. In all experiments axenically grown, viable, carefully washed nematodes were used.[7] To prevent the organisms from consuming the homogenate, the nematodes were poisoned in 10 mM KCN for 1 h and, after five washings, used directly in the binding assays. Table 2 shows that binding took place and that it could be partially inhibited (50–80 %) by *N*-acetyl-D- galactosamine. Although differences between samples of both fungi and nematodes were inevitable, in all cases a marked reduction in binding took place in the presence of *N*-acetyl-D-galactosamine.

Trypsin treatment (final concentration 10 mg/ml in the same buffer as used earlier, 1 h at 37 °C) of the nematode surface prior to binding studies resulted in a reduction in binding capacity (67 %) indicating that glycoproteins are possibly present on the nematode-surface.

Enzyme modification of the nematode surface by treatment with either β-galactosidase or β-1,3-glucanase increased subsequent binding (two to three times) of labelled homogenate. Treatment with dextranase and neuraminidase, however, only marginally altered the binding pattern.

These experiments provide further evidence for the existence of a trap lectin specific for *N*-acetyl-D-galactosamine. In addition, the results indicate the presence of a glycoprotein on the nematode with carbohydrate moieties sensitive to enzymatic attack. Further studies on this system are now in progress.

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