THE CHEMICAL NATURE OF THE AMPHIDIAL AND " EXCRETORY " SYSTEM SECRETIONS OF *HETERODERA SCHACHTII* (NEMATODA : HETERODERIDAE) MALES

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The amphids are supposed to be the main chemosensilla of the nematode head. The dendritic nerve extensions of the amphidial neurons are surrounded by secretions of the amphidial gland cells (Wright, 1983). The secretions may protect the nerve dendrites against microbial attack (Aumann & Wyss, 1989). In spite of a recent proposal (Bird *et al.,* 1988) the function of the so-called " excretory " system and of its gland cell secretions is yet unknown. The chemical characterization of the secretions may aid in the determination of their functions.

Previous studies showed that the amphidial secretions of the plant-parasitic nematode *Heterodera schachtii* are composed of glycoproteins with terminal galactose units (Aumann, 1989). Several lectins with different carbohydrate specificities bind to the amphidial and " excretory " system secretions of this (Aumann & Wyss, 1989) and other nematode species (Jansson, 1987). The carbohydrates may be bound to the protein backbone either N-glycosidically via N-acetylglucosamine and asparagine or O-glycosidically via N-acetylgalactosamine and serine or threonine. Fetuin, a blood glycoprotein, con-

tains both linkeage types (Reuter *et al.*, 1988). The molecules may be composed of subunits that are joined together by disulphide bridges (Slomiany *et al.*, 1989).

The aim of the present study was to test *i)* the type of protein-carbohydrate linkeage and *ii)* the occurrence of disulphide-linked subunits, in the amphidial and " excretory " system secretions of *H. schachtii* males. For this purpose the effect of different reagents on lectin binding to the secretions was observed by fluorescence microscopy. The presence of N-glycans was tested by incubating the nematodes with the enzyme N-glycosidase F, which cleaves the linkeage between N-acetylglucosamine and asparagine, whereas the presence of O-glycans was examined by treating the nematodes with trifluoromethanesulphonic acid, which removes, under the experimental conditions used, all carbohydrates except those N-acetylglucosamine residues bound to asparagine. The occurrence of disulphide-linked subunits was tested by treating the nematodes with 6 mol/l guanidine hydrochloride plus 100 mmol/l dithiothreitol.

Materials and methods

Nematodes

Males of *H. schachtii* were obtained from monoxenic cultures of oilradish *(Raphanus sativus* var. *oleifera)* cv. Nemex (Saatzucht P.H. Petersen, Lundsgaard, Germany) grown at 25 ± 2 °C in a nutrient agar medium (Knop, 1865; one fifth normal concentration) supplemented with 2 % sucrose and Murashige and Skoog vitamin solution (Sigma, Deisenhofen, Germany).

Glycoprotein-modifying reagents

N-Glycosidase F (EC 3.2.2.18; Boehringer, Mannheim, Germany) was applied for 22.5 h at 37 °C in microcentrifuge tubes in a concentration of 40 U/ml in Na-phosphate buffer, pH 7.2 (PB). In order to enhance the access of the enzyme to the protein backbone (Aumann, 1989), the nematodes had previously been incubated for 70.5 h in ultrapure water (Merck, Darmstadt, Germany) at 20 °C and then washed three times with PB. Concentrated trifluoromethanesulphonic acid (Aldrich, Steinheim, Germany) was mixed 2:1 with ultrapure water and then applied for 1 h at 20 °C in glass staining dishes. Guanidine hydrochloride (6 mol/l) plus dithiothreitol (0.1 mol/l; both from Sigma) in 0.135 mol/l Tris-HCl, pH 8.0, was applied for 44 h at 4 °C in microcentrifuge tubes. All treatments with glycoprotein-modifying reagents were performed under constant shaking.

Lectin binding

After the nematodes had been washed up to three times with phosphate buffered saline, pH 6.8 (PBS), they were incubated for 30 min at 20 °C in the dark with 200 µg/ml of the tetramethylrhodamine isothiocyanate (TRITC)-conjugated lectin from *Canavalia ensiformis* (Con A; from Sigma), which belongs to the glucose/mannose specificity group. As an additional marker for the occurrence of N-glycans that may not have been removed by an incubation with N-glycosidase F, the TRITC-conjugated lectin from *Triticum vulgare* (WGA; also from Sigma), which belongs to the N-acetylglucosamine specificity group, was applied under the same conditions after the trifluoromethanesulphonic acid treatment. Lectin binding sites were observed by fluorescence microscopy as described by Aumann and Wyss (1989). All experiments were performed three times.

Results

Table 1 shows that of the three treatments applied only trifluoromethanesulphonic acid had reduced the percentage of *H. schachtii* males with lectin binding sites at the amphidial secretions. Compared with the controls neither N-glycosidase F nor guanidine hydrochloride plus dithiothreitol affected the binding sites of the lectin Con A. All nematodes treated with these reagents and all control nematodes tested showed lectin binding sites at the amphidial secretions.

No binding sites of Con A and WGA at the " excretory " system secretions were observed after a nematode treatment with trifluoromethanesulphonic acid. In the water controls, 46.7 % of the nematodes showed Con A binding sites and 6.7 % showed WGA binding sites at these secretions. Con A bound to 40 % of the secretions in the Tris-HCl controls, whereas 77.1 % of the nematodes showed Con A binding sites in the sodium phosphate controls (Table 1).

Discussion

Most animal cell surface glycoproteins and various secreted and intracellular glycoproteins have been identified as N-glycans (Cummings *et al.*, 1989). O-Glycans are the major constituents of mucus and are also found in certain cell membrane-associated molecules (Hilkens *et al.*, 1992). According to Plummer *et al.* (1984), N-glycosidase F cleaves N-glycans between N-acetylglucosamine and asparagine. O-Glycosidic linkeages between N-acetylgalactosamine and serine or threonine are not affected. Because of the liberation of virtually all N-linked oligosaccharide chains the enzyme is now used in several standard procedures for the deglycosylation of N-glycans. Within 2.5 h at 0 °C or 1 h at 25 °C trifluoromethanesulphonic acid removes all mannose and most of the galactose residues from fetuin except those N-acetylglucosamine residues bound to asparagine. Under these conditions the amino acid composition remained almost unchanged. Similar results were obtained with other glycoproteins (Edge *et al.*, 1981). It may be concluded that, under the conditions applied to *H. schachtii* (1 h at 20 °C), the protein backbone of the secretions and any possibly occurring asparagine-bound N-acetyl-

Table 1. *The effects of glycoprotein-modifying reagents on lectin binding to the amphidial and " excretory " system secretions of* Heterodera schachtii *males.*

Treatment	n	No. (%) of males with lectin binding sites			
		Amphids		" Excretory " system	
		Con A	WGA	Con A	WGA
N-Glycosidase F	35	35 (100)	–	21 (60.0)	–
Sodium phosphate, pH 7.2	35	35 (100)	–	27 (77.1)	–
Trifluoromethanesulphonic acid	30	4 (13.3)	6 (20.0)	0	0
Water	30	30 (100)	30 (100)	14 (46.7)	2 (6.7)
Guanidine-HCl plus dithiothreitol	40	40 (100)	–	35 (87.5)	–
Tris-HCl, pH 8.0	40	40 (100)	–	16 (40.0)	–

glucosamine residues should have remained unaffected. The experiments with N-glycosidase F and trifluoromethanesulphonic acid thus indicate that the amphidial and " excretory " system secretions of *H. schachtii* males are composed exclusively of O-glycans.

According to Aumann and Wyss (1989), Con A (glucose/mannose specificity group) and WGA (N-acetylglucosamine specificity group) and the lectins from *Helix pomatia* and *Arachis hypogaea* (galactose/N-acetylgalactosamine specificity group) specifically bind to the amphidial secretions of *H. schachtii* males. The lectin from *A. hypogaea* also specifically bound to the " excretory " system secretions. Table 1 shows that, in contrast to previous observations (Aumann & Wyss, 1989), Con A bound to the " excretory " products, which may have been caused by an increased accessibility of internal Con A binding sites after the pre-incubation of nematodes with ultrapure water (Aumann, 1989). The variability of Con A binding to the " excretory " system secretions (Table 1) may be explained by the relatively small size of the " excretory " pore and by its variable position in specimens that had been fixed on microscopic slides. In summary, these data suggest that mannose or glucose, N-acetylglucosamine, and galactose and/or N-acetylgalactosamine are components of the amphidial secretion oligosaccharide chains, whereas the oligosaccharides of the " excretory " system secretions may contain galactose and/or N-acetylgalactosamine residues. Furthermore, as N-glycans do not seem to occur in the amphidial and " excretory " system secretions (Table 1), these monosaccharide residues may be components of O-glycans. This points to an unusual composition of *H. schachtii* O-glycans, since, in contrast to N-glycans (Kornfeld & Kornfeld, 1985), O-glycans usually do not contain mannose residues (Schachter & Brockhausen, 1989).

Human gastric mucus glycoproteins appear to be composed of four subunits linked together via disulphide bridges (Slomiany *et al.*, 1989). Using the guanidine hydrochloride plus dithiothreitol method, subunits were obtained from several mucous glycoproteins (Carlstedt *et al.*, 1982; Meyer, 1983; Carlstedt & Sheehan, 1984). This method did not affect the amphidial and " excretory " system secretions of *H. schachtii* males (Table 1), indicating that they are not composed of disulphide-linked subunits.

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PLESIOROTYLENCHUS TRUNCATUS (SHER, 1964) N. COMB. (NEMATA : HOPLOLAIMIDAE)

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