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Characterization and Localization of Saccharides on the Head Region of Four Populations of the Potato Cyst Nematode *Globodera rostochiensis* and *G. pallida*

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Abstract: N-acetylglucosamine, galactose, N-acetylgalactosamine and mannose and (or) glucose were present on specimens of two populations of *Globodera rostochiensis* and two of *G. pallida* representing four different pathotypes. Individuals within the pathotypes varied in the amounts of some of the saccharides present. The Pa_1 population differed from the other populations in the presence on all individuals of N-acetylgalactosamine and the absence of extensive residues of mannose/glucose. TEM studies showed that N-acetylglucosamine and mannose/glucose were present on the exudate from the amphids of juveniles from the Ro_1 population.

Key words: amphidial exudate, N-acetylglucosamine, galactose, N-acetylgalactosamine, mannose, glucose, fluorescence, microscopy, electron microscopy, rhodamine, ferritin, pathotypes, potato cyst nematode.

Saccharides are present on the surface of plant and animal parasitic and free-living nematodes (1,5,7,14), and it has been postulated (14) that these surface carbohydrates may play an important role in the interaction between nematodes and their hosts. The occurrence of saccharides on the cuticle of invasive juveniles (J2) of *Globodera rostochiensis* (Woll.) Behrens and *Globodera pallida* (Stone) Behrens is examined here.

Materials and Methods

Nematodes: Hatched J2 of populations representing *G. rostochiensis* pathotypes Ro_1 and Ro_5 and *G. pallida* pathotypes Pa_1 and Pa_2, as defined by Kort et al. (4), were obtained as previously described (3). Cysts were presoaked for 7 days in tap water and then in potato root diffusate. Juveniles that hatched within 24 hours were used for lectin binding assays.

Chemicals: Rhodamine conjugates of the lectins Concanavalin A (Con A), *Dolichos biflorus* agglutinin (DBA), *Limax flavus* agglutinin (LFA), *Limulus polyphemus* agglutinin (LPA), peanut agglutinin (PNA), *Ricinus communis* agglutinin (RCA 120), *Ulex europaeus* agglutinin (UEA), and wheat germ agglutinin (WGA) were obtained from Sigma (Poole, Dorset, U.K.), E-Y Laboratories (San Mateo, Calif.), or Vector Laboratories (Burlingame, Calif.). For tests of binding specificity α-methylmannoside, D-galactose, and N-acetylgalactosamine were obtained from BDH Ltd. (Poole, Dorset). A crude mixture of oligomers of N-acetylglucosamine was donated by Dr. D. C. Kilpatrick of the Blood Transfusion Unit, Edinburgh Royal Infirmary. Thereafter, the oligomers were prepared by the method of Stirling (9). Ferritin conjugates of WGA and Con A were obtained as solutions from Sigma Ltd. or E-Y Laboratories. Glutaraldehyde for electron microscopy was obtained from BDH Ltd. as a 25% aqueous solution.

Lectin binding assay: Approximately 200 J2 were washed three times in cold phosphate buffered saline (PBS), pH 7.4, and incubated with 50 μl lectin-rhodamine conjugate (100 μg/ml in PBS) for 2 hours at 4 C. In tests with LFA and LPA Tris buffered saline (TBS), pH 7.4, was used instead of PBS. As a control, the appropriate competing saccharide was included at a concentration of 200 mM. Treated J2 were washed three times and mounted on a glass slide in p-phenylenediamine-PBS-glycerol (6). Each lectin and pathotype was tested in binding assays at least four times except for LFA and LPA which were tested twice only. Nematodes exposed to lectins and the appropriate controls were examined using incident fluorescence microscopy.

Transmission electron microscopy: Freshly

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TABLE 1. Binding of lectin-rhodamine conjugates to two populations of pathotypes of *Globodera rostochiensis* and two of *G. pallida*.

Lectin	Specific sugar	*G. rostochiensis*		*G. pallida*	
WGA	Oligomers of N-acetylglucosamine	Ro_1 +	Ro_5 +	Pa_1 +	Pa_2 +
Con A	Mannose/glucose	+−+++	+−+++	+	+−+++
PNA/RCA 120	Galactose	±	±	±	±
DBA	N-acetylgalactosamine	±	±	+	±
LFA	N-acetylneuraminic acid	−	−	−	−
LPA	N-acetylneuraminic acid	−	−	−	−
UEA	Fucose	−	−	−	−

+−+++ Present in variable amounts, some individuals having extensive labelling at the head.
± Some individuals labelled, others unlabelled.
− No individuals labelled.

hatched J2 were washed three times in PBS before being fixed with 2% glutaraldehyde in PBS at ca. 20 C. The fixed J2 were then rinsed with PBS three times (5 minutes each time) before being placed in 0.1 ml Con A or WGA-ferritin in 0.3 ml PBS for 30 minutes at 20 C. After further rinsing J2 were postfixed with 1% osmium tetroxide in PBS for 30 minutes, rinsed again, and decapitated. The heads were embedded in small blocks of 1% agar and dehydrated in an ethanol series followed by two changes of polypropylene oxide. Finally, the blocks containing the heads were placed in E-mix MED resin (Emscope Laboratories, Ashford, Kent) at 35 C for 2 hours before being poured into flat embedding molds where the resin was polymerized overnight at 60 C.

RESULTS

Except for LFA, LPA, and UEA, all the lectins bound to some part of the head of freshly hatched J2 of each potato cyst nematode pathotype (Table 1), indicating the presence of N-acetylglucosamine, mannose and (or) glucose, galactose, and N-acetylgalactosamine and the absence of fucose and N-acetylneuraminic acid. Lectin binding was shown to be specific, as it was blocked by the presence of the appropriate competing saccharide. Differences in the intensity and proportion of individuals within pathotypes labeled with PNA or RCA 120 and DBA, which has a greater affinity for N-acetylgalactosamine, suggest that both galactose and N-acetylgalactosamine are present.

WGA bound in a uniform manner to small areas of the heads of all J2 examined (Fig. 1a, b). TEM studies showed that Con A and WGA-ferritin bound to an amphidial exudate from juveniles of a population of Ro_1 (Fig. 1c). Con A also bound to the amphidial openings of every nematode, and larger areas of the head were often labelled on some individuals of Ro_1, Ro_5, and PA_2. Extensive labelling of the head of J2 of Pa_1 by Con A was never found. RCA 120 and PNA bound locally with varying degrees of intensity on the head of some individuals of all populations but not to others.

Juveniles of the Pa_1 population were distinguished from those of other pathotypes because every individual was labelled by DBA. Only a small proportion of the other pathotypes gave weakly positive results.

DISCUSSION

Our results provide the first evidence for the occurrence of saccharides on the surface of the potato cyst nematodes *G. rostochiensis* and *G. pallida*. The presence of the same saccharides (N-acetylglucosamine, glucose/mannose and galactose/N-acetylgalactosamine) has been previously reported on *Caenorhabditis elegans, C. briggsae, Meloidogyne javanica, M. incognita,* and *Xiphinema index* among others (5,7,8,13). These saccharides were not generally distributed over the body surface of potato cyst nematode J2 but appeared to be confined mainly to small areas of the head. Ferritin conjugates of WGA and Con A bound to an exudate from the amphids of juveniles from a population of Ro_1, and it seems likely that these sites are bound on other pathotypes as well. In contrast,

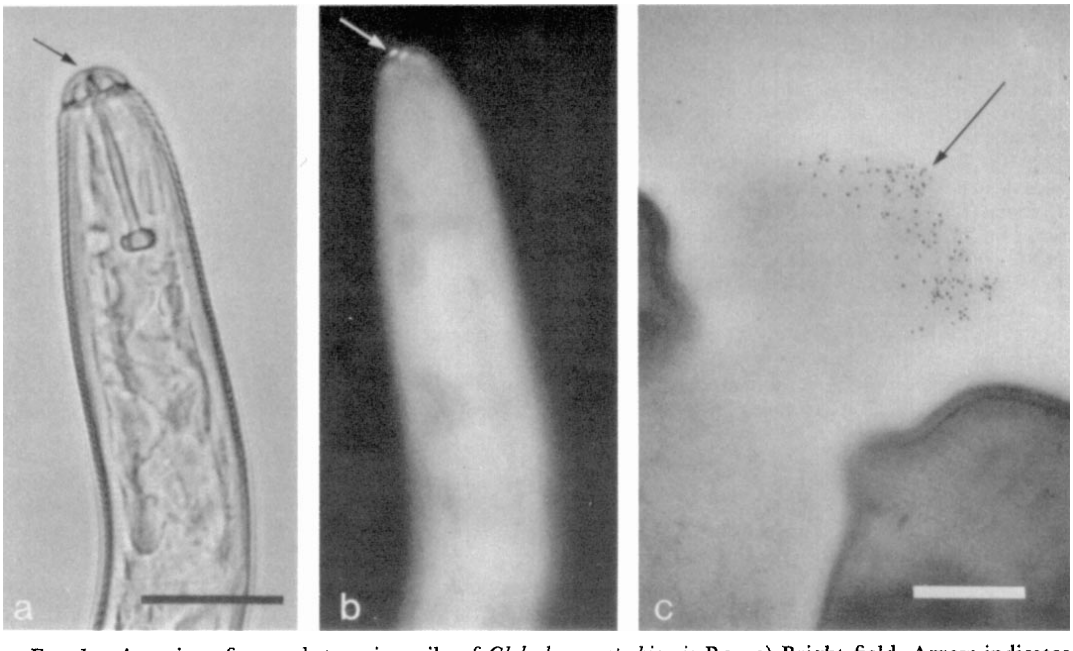

FIG. 1. Anterior of second-stage juvenile of *Globodera rostochiensis* Ro_1. a) Bright field. Arrow indicates where WGA-TRITC binds. Bar = 20 μm. b) Same nematode as in Figure 1a under incident fluorescence specific for rhodamine. Arrow indicates two spots over the amphids. The body is rendered fluorescent by post-fixation with 1% glutaraldehyde in PBS. Bar = 20 μm. c) Exact location (arrow) of binding on nematode in Figure 1a. WGA-ferritin conjugate binds to amphidial exudate. Bar = 200 nm.

McClure and Zuckerman (5) found no labelling of the amphidial exudate of *C. elegans* but heavy labelling of other parts of the head. Spiegel et al. (8) also found that ferritin-labelled reagent specific for sialic acid bound to the amphid apertures of *X. index* among other places.

The role of the amphids is unknown. Zuckerman (12) proposed that amphids act as chemoreceptors during host finding, while it has also been suggested that the amphidial exudate may flush and protect these sensory organs (D. L. Trudgill, pers. comm.). Endo (2) demonstrated that the feeding plug produced by *Heterodera glycines* in soybean roots had continuity with secretions that appeared to flow from the amphidial canals, the openings of the inner labial receptors, and the stylet vestibule. He concluded that the amphidial gland was one possible source of the electron-dense deposits that formed the plug. A further intriguing suggestion is that these saccharide residues, which clearly differ between individuals and some populations, could play a role in eliciting a resistant response within the plant. It is known that glycoproteins rich in mannose and galactose extracted from *Cladosporium fulvum* acted as elicitors of phytoalexin production in tomato (11).

Trudgill (10) has argued convincingly that many English populations of *G. pallida* are heterogeneous with regard to virulence genes. If the differences we have described are related to virulence, then the way is open for the production of monoclonal antibodies and the development of tests that can measure precisely the virulence of a nematode population to a given source of resistance.

LITERATURE CITED

1. Clark, N. W. T., M. Philipp, and R. M. E. Parkhouse. 1982. Non-covalent interactions result in aggregation of surface antigens of the parasitic nematode *Trichinella spiralis*. Biochemical Journal 206:27–32.

2. Endo, B. Y. 1978. Feeding plug formation in soybean roots infected with soybean cyst nematode. Phytopathology 68:1022–1031.

3. Forrest, J. M. S., and L. A. Farrer. 1983. The response of eggs of the white potato cyst nematode *Globodera pallida* to diffusate from potato and mustard roots. Annals of Applied Biology 103:283–289.

4. Kort, J., H. Ross, H. M. Rumpenhorst, and A.

R. Stone. 1977. An international scheme for identifying and classifying pathotypes of potato cyst-nematodes *Globodera rostochiensis* and *G. pallida.* Nematologica 23:333–339.

5. McClure, M. A., and B. M. Zuckerman. 1982. Localization of cuticular binding sites of Concanavalin A on *Caenorhabditis elegans* and *Meloidogyne incognita.* Journal of Nematology 14:39–44.

6. Platt, T. L., and A. F. Michael. 1983. Retardation of fading and enhancement of intensity of immunofluorescence by p-phenylenediamine. Journal of Histochemistry and Cytochemistry 31:840–842.

7. Spiegel, Y., E. Cohn, and S. Spiegel. 1982. Characterization of sialyl and galactosyl residues on the body wall of different plant parasitic nematodes. Journal of Nematology 14:33–39.

8. Spiegel, Y., W. M. Robertson, S. Himmelhoch, and B. M. Zuckerman. 1983. Electron microscope characterization of carbohydrate residues on the body wall of *Xiphinema index.* Journal of Nematology 15: 528–534.

9. Stirling, J. L. 1974. Human N-acetyl-beta-hexosaminidases: Hydrolysis of N,N′-diacetylchitobiose by a low molecular weight enzyme. FEBS Letters 39: 171–175.

10. Trudgill, D. L. 1985. Potato cyst nematodes: A critical review of the current pathotyping scheme. EPPO Bulletin, in press.

11. de Wit, P. J. G. M., and P. H. M. Roseboom. 1980. Isolation, partial characterization and specificity of glycoprotein elicitors from culture filtrates, mycelium and cell walls of *Cladosporium fulvum* (syn. *Fulvia fulva*). Physiological Plant Pathology 16:391–408.

12. Zuckerman, B. M. 1983. Hypotheses and possibilities of intervention in nematode chemoresponses. Journal of Nematology 15:173–182.

13. Zuckerman, B. M., and I. Kahane. 1983. *Caenorhabditis elegans*: Stage-specific differences in cuticle surface carbohydrates. Journal of Nematology 15:535–538.

14. Zuckerman, B. M., and H.-B. Jansson. 1984. Nematode chemotaxis and possible mechanisms of host/prey recognition. Annual Review of Phytopathology 22:95–113.