Journal of Nematology 18(4):570–574. 1986.
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Fluorescent and Ferritin Labelling of Cuticle Surface Carbohydrates of *Caenorhabditis elegans* and *Panagrellus redivivus*

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Abstract: *Caenorhabditis elegans* and *Panagrellus redivivus* were investigated for surface carbohydrates using fluorescent-labelled and ferritin-labelled lectins. Rhodamine-labelled Concanavalin A was specifically located in the cephalic region of both species. Rhodamine-labelled wheat germ agglutinin was located over the entire cuticle of *P. redivivus* but was absent on *C. elegans.* Rhodamine-labelled peanut agglutinin and *Limax flavus* agglutinin did not label nematodes of either species. Galactose and sialic acid were not detected on either species, whereas mannose-glucose residues were specifically localized in the head areas of both species. No detectable *N*-acetylglucosamine occurred on *C. elegans,* but it was evenly distributed over the cuticle surface of *P. redivivus.*
Key words: *Caenorhabditis elegans,* cuticle, fluorescence, microscopy, lectin, *Panagrellus redivivus,* surface carbohydrate, transmission electron microscopy.

Carbohydrates localized on nematode cuticles appear to be important in recognition phenomena in which nematodes are involved (14). Carbohydrates (e.g., mannose, glucose, *N*-acetylgalactosamine, sialic acid) have been reported on the cuticle surface of *Globodera* sp., *Meloidogyne* sp., *Xiphinema* sp., and other nematode species (1,3,8,11,12). In some cases the carbohydrates have been localized in the head and tail regions, and in other cases they were evenly distributed over the entire cuticle.

Recognition on the molecular level between nematodes and nematophagous fungi appears to include a lectin–carbohydrate interaction where the lectin, located on the infective structures of the fungus, binds to a specific carbohydrate on the nematode surface (6,9,10). Most of these interactions imply that the carbohydrates are distributed over the entire cuticle, since adhesion of nematodes to the fungal traps takes place nonselectively. The only known exception is the fungus *Drechmeria coniospora* where the conidia adhere only to the head and tail regions of most different species of nematodes tested (5,6).

The importance of carbohydrates in nematode chemotaxis was suggested by Zuckerman (13) and Zuckerman and Jansson (14). By treating the nematodes with certain carbohydrate-splitting enzymes or corresponding sugar-specific lectins, it was possible to interfere with nematode chemotactic behavior. Sialic acid and mannoside residues were found in such experiments to be vital for chemotaxis of *Caenorhabditis elegans* and *Panagrellus redivivus* (4,7); mannose was also found important for *Trichostrongylus colubriformis* chemotaxis (1). Our objectives were to visualize and thereby establish the distribu-

Received for publication 6 February 1986.
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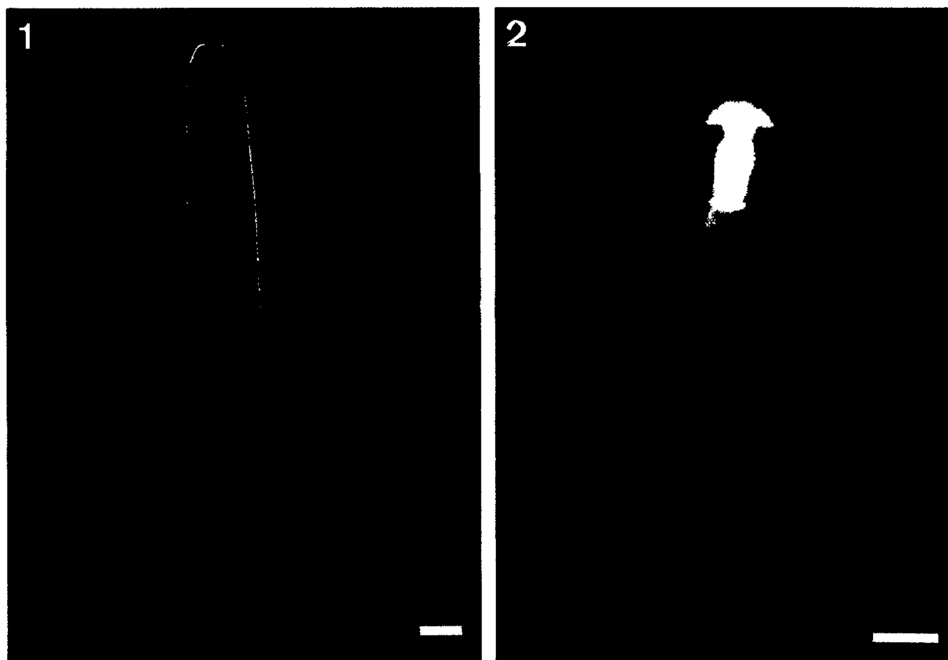

FIGS. 1, 2. Photomicrographs of nematodes treated with TRITC-labelled lectins. 1) *Panagrellus redivivus* labelled with TRITC-WGA over the entire cuticle. 2) *Caenorhabditis elegans* specifically labelled with TRITC-Con A in the cephalic region. Bars = 10 μm.

tion of several nematode cuticle surface carbohydrates using fluorescent-labelled and ferritin-labelled lectins.

MATERIALS AND METHODS

C. elegans and *P. redivivus* were cultured axenically at 22 C in a medium consisting of 3 g yeast extract, 4 g soy peptone, 50 mg hemiglobin, and 90 ml water (8). Before the experiments, live nematodes were separated from dead by Baermann funnel; the live nematodes were washed in 5 mM tris buffer, pH 7.2, 3–5 times by centrifugation at 5,900 *g*.

Both fluorescein isothiocyanate (FITC) and rhodamine (TRITC) conjugates of *Limax flavus* agglutinin (LFA), peanut agglutinin (PNA), wheat germ agglutinin (WGA), and Concanavalin A (Con A) were tested for the fluorochrome trials. Nonlabelled nematodes gave a strong autofluorescence with the filter for FITC but not with the TRITC filter. Therefore, only results with TRITC-labelled nematodes are presented. All labelled lectins were purchased from EY Laboratories, San Mateo, California.

Medium-free nematodes were treated with each labelled lectin at 25 μg/ml in 5 mM tris buffer, pH 7.2, for 20 minutes. After washing 3–5 times with the buffer, labelled nematodes were examined under a Nikon fluorescence microscope equipped with FITC and TRITC filters. Nematodes were heat-killed before photographs were taken. No substantial differences in labelling were observed between living and heat-killed nematodes.

A control test was performed by first exposing nematodes to the lectin conjugate, washing them in buffer, and then incubating with the specific sugar (for Con A: 0.1 M α-methyl-D-mannoside; for WGA: 0.1 M *N*-acetyl-D-glucosamine). Specific labelling was indicated by a reduction of intensity of fluorescence through competitive displacement of the lectin. A second control was established where, after pretreatment of the lectins with the specific sugar haptens before exposure to the nematodes, a lack of TRITC-labelling was shown.

P. redivivus were also labelled with WGA-ferritin and LFA-ferritin conjugates (EY Laboratories) to attempt visualization of the distribution of *N*-acetylglucosamine and sialic acid residues, respectively, by trans-

TABLE 1. Labelling of *Caenorhabditis elegans* and *Panagrellus redivivus* with rhodamine conjugated lectins.

Lectin	Specific sugar	*C. elegans*	*P. redivivus*
WGA	*N*-acetylglucosamine	No label	General label over entire cuticle
Con A	Glucose-mannose	Localized to head region	Localized to head region
PNA	Galactose *N*-acetylgalactosamine	No label	No label
LFA	Sialic acid	No label	No label

mission electron microscopy (TEM). Labelling and processing for electron microscopy was as described by Zuckerman et al. (15). Random sections through nematodes were observed for the *N*-acetylglucosamine residues, and sections through heads exposing the pores of the anterior portions of the chemosensilla were examined for sialic acid residues. Photomicrographs were taken on a JEOL 100S electron microscope operated at 80 kV.

RESULTS

TRITC-labelled WGA was the only lectin that showed a difference in binding between the two nematode species. The entire cuticle of *P. redivivus* was labelled (Fig. 1), whereas virtually no label occurred on the cuticle of *C. elegans*. As in most of the treatments, the buccal cavity of *C. elegans* was heavily labelled. WGA binds specifically to *N*-acetylglucosamine, indicating its abundant presence on *P. redivivus* and sparse occurrence or absence on *C. elegans*.

PNA binds specifically to galactose residues and primarily to the disaccharide galactose-*N*-acetylgalactosamine. TRITC-labelled PNA did not bind to the cuticle of any of the nematodes and was visible only in the buccal cavity. Similar negative binding occurred with the sialic acid specific lectin LFA (Table 1).

Con A was the only lectin that showed specific localization on the two nematode species. TRITC-Con A bound specifically to the tip of the head of both *C. elegans* (Fig. 2) and *P. redivivus*, indicating the presence of mannose and (or) glucose in the cephalic region (except for the buccal cavity and part of the esophagus). Results of the TRITC-labelling trials are summarized in Table 1.

Photomicrographs taken by TEM from random showed that the WGA-ferritin conjugate was distributed over the entire surface of the cuticle glycocalyx of *P. redivivus* (Fig. 3) but was not detected on *C. elegans*. These observations support those of the TRITC-labelling, indicating uniform distribution of *N*-acetylglucosamine over the surface of the cuticle of *P. redivivus*. Extensive examination of sections through the pores of the anterior sensilla failed to reveal labelling by the LFA-ferritin conjugate suggesting the absence of sialic acid, consistent with the results of the TRITC trials.

DISCUSSION

The fluorescence observed in the buccal cavity and (or) the anterior portion of the esophageal lumen probably resulted from ingestion of TRITC-labelled lectin by the nematodes. Therefore, this fluorescence does not necessarily indicate the specific labelling of carbohydrates on the buccal or esophageal inner surfaces.

The localization of Con A receptors in the head region of the two nematodes was not unexpected. In *C. elegans* the localization of mannose-glucose was previously shown using a hemocyanin–Con A conjugate (8). To our knowledge, this is the first report of the localization of a Con A receptor on *P. redivivus*. It was shown in earlier experiments that treatment of the nematodes with Con A and mannosidase, but not glucosidase, inhibited the chemotactic ability of both *C. elegans* and *P. redivivus* (4,7). Thus, mannose seems to be important in nematode chemotaxis and appears to be localized close to the cephalic sensory structures. Whether these mannose residues are more closely bound to the nematode surface or emanate from sensilla exudates, as previously suggested (13), is not known.

Sialic acid, the other carbohydrate important in nematode chemotaxis (4,7) and in the adhesion of conidia of the nemato-

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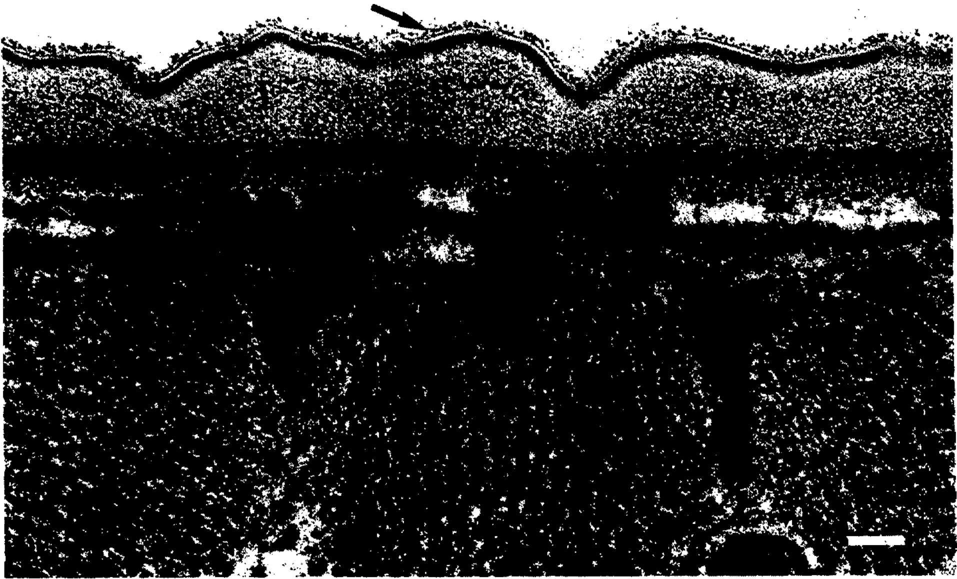

FIG. 3. Transmission electron micrograph of the cuticle surface of a *Panagrellus redivivus* nematode labelled with ferritin-WGA (arrow). Bar = 0.1 μm.

phagous fungus *D. coniospora* to the cephalic region of the nematodes (6), was not detected using TRITC-labelled LFA or the LFA-ferritin conjugate. Lack of labelling may be caused by steric inaccessibility of the nematode sialic acid molecules to the lectin, or there may be too few sialic acid molecules to discriminate from background using this technique. Reports of the presence of sialic acid on nematodes are based on biological data (4–7) and on one report of observing sialyl residues (12); we did not manage to visualize sialic acid on either nematode, *C. elegans* or *P. redivivus,* in our experiments. We are currently attempting to develop a highly sensitive microchemical test to confirm the occurrence of sialic acids on nematodes.

A situation similar to the lack of binding of LFA conjugates may occur with the TRITC-labelled PNA. PNA binds to galactose residues. *N*-acetylgalactosamine has been demonstrated on the cuticle of *P. redivivus* (2,9) and other nematode species (1,3,11). Forrest and Robertson (3), using rhodamine-labelled PNA and *Ricinus communis* agglutinin to locate galactose residues on the surface of *Globodera* spp., reported variable results with some specimens labelled and others remaining unlabelled. No cuticle labelling with TRITC-PNA occurred on either *C. elegans* or *P. redivivus* in the current study. These differing results indicate that nematode surface carbohydrates may be sterically inaccessible to certain lectins or that some carbohydrate residues may be too few to visualize with the technique used.

The TRITC-labelled WGA was the only lectin that showed a difference between the two nematode species tested. WGA binds specifically to *N*-acetylglucosamine; therefore, the pattern of binding indicates that this carbohydrate occurs over the entire cuticle of *P. redivivus* but is not detectable or is absent on *C. elegans.* The presence of *N*-acetylglucosamine on *C. elegans* was previously shown with a radioassay using ^{125}I-labelled WGA (15), indicating that fluorescent-labelling of lectins is less sensitive than using radiolabels.

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