Localization of Cuticular Binding Sites of Concanavalin A on *Caenorhabditis elegans* and *Meloidogyne incognita*[1]

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Abstract: Utilizing a Concanavalin A (Con A)-hemocyanin conjugate, the majority of cuticular Con A binding sites were shown to be localized on the head region of *Caenorhabditis elegans* and *Meloidogyne incognita.* Secretions which apparently emanated from the amphids and inner labial papillae did not label. *Key words: Caenorhabditis elegans, Meloidogyne incognita,* Concanavalin A, cuticle, scanning electron microscopy.
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Lectins and other proteins that bind to specific molecules or molecular radicals have been used extensively to examine and characterize certain aspects of cell surface phenonema and are now coming into use in nematology. The development of these labelling techniques is leading to a more precise understanding of the nematode surface and, ultimately, the role which these strategically placed surface molecules play in vital interactions between the organism and its environment.

Hemocyanin, which forms particles of distinctive size and shape that can be readily identified under the electron microscope, is an ultrastructural marker which has been used to identify lectins bound to their target sites (4,5). The size of the hemocyanin particle ($\sim 10 \times 35$ nm) enables visualization by scanning electron microscopy (SEM), thereby facilitating examination of the distribution of specific oligosaccharides on the nematode surface.

Previously, utilizing ^{125}I labelled Concanavalin A (Con A), wheat germ agglutinin and *Ricinus communis* agglutinin, residues of galactose, n-acetylglucosamine, α-methyl-D-mannoside or other mannosides were demonstrated on the surface of *Caenorhabditis elegans* (7). Comparison with the results of similar studies on mycoplasma cell membranes (1) suggested that these carbohydrate residues were present in much smaller amounts over the total nematode surface than on mycoplasma mem-

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branes, possibly indicating an uneven distribution.

The current study was undertaken to determine the distribution of mannose and mannoside residues on the surface of *C. elegans* and *Meloidogyne incognita* utilizing a Con A-hemocyanin conjugate for visualization of binding sites by SEM.

MATERIALS AND METHODS

Processing: *C. elegans* was cultured axenically at 22 C in a medium consisting of 4 g yeast extract, 3 g soy peptone, 50 mg hemiglobin (Calbiochem, La Jolla, California Cat. No. 3746) and 90 ml of water. This medium was made by dissolving the heme in 0.1 N NaOH then sterilizing the solution by passage through a 0.22 μm filter. The other ingredients were heat sterilized separately and the two fractions combined.

Specimens of *C. elegans* were selected in a different way for each of four scanning electron microscope experiments (SEM). In the first three experiments, young or old hermaphrodites were hand picked into Tris buffer (pH 7.3), and each group processed separately for examination. In the final run a mixture of nematodes of all age groups was processed. The approximate ages of *C. elegans* from mixed culture were established based on nematode size, shape of the vulva lips, and the appearance of the vulval aperture.

Eggs of *M. incognita* were harvested from roots of greenhouse-grown chili peppers, *Capsicum frutescens* (2), and freshly hatched, infective, second-stage larvae were concentrated into .067 M phosphate buffer, pH 7.3, by centrifugation at 3,000 × g. Further treatment of both *C. elegans* and *M. incognita* was achieved by drawing the nematodes into a chamber (3) where they were labelled with Con A/hemocyanin and processed for SEM as follows: The nematodes were washed six times with Tris buffer (pH 7.3), incubated in Con A (200 μg/ml) in Tris buffer for 15 min at 4 C, washed six times with buffer, incubated with hemocyanin (1 mg/ml) in 3% NaCl for 15 min at 4 C, washed six times with buffer, and incubated in 0.1 M galactose in Tris buffer for 15 min at 4 C. The galactose wash, which was to remove nonspecifically bound Con A, was changed several times during the incubation period. The nematodes were then washed six times with Tris buffer and fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.2) overnight. This step was followed by six washes in phosphate buffer, post fixation in 1% osmium tetroxide in phosphate buffer for 1 h, and six washes in distilled water, after which the specimens were dehydrated in an acetone series in 10% increments from 10 to 100%, with two changes in 100% (15 min/change). The nematodes were then critical point dried, mounted on stubs, and coated wth 15 nm gold/palladium (60/40) in a sputter coater at 5 C. The nematodes were viewed and photographed on an ISI DS-130 SEM at 40 kV.

The possibility of nonspecific binding of hemocyanin to the cuticle was evaluated by incubating the nematodes in hemocyanin without prior incubation in Con A. These nematodes were then processed for scanning electron microscopy in the same manner as those in the Con A series.

Scans of each nematode were concentrated on several body areas: the head, the vulva and the surrounding midbody region, the anus, and the tail. Occasional scans were also made of the excretory pore, other areas of the midbody, and the phasmids. In several trials ligand-induced clustering of hemocyanin molecules occurred, but aggregation did not interfere with the visualization of single hemocyanin molecules.

Quantitation of labelling: Evaluation of the degree of labelling was based on the number of hemocyanin molecules visualized per square micron as follows: heavy, 11–25 molecules; moderate, 5–10 molecules; light, 2–4 molecules; sparse, 1 molecule or less. Generally no hemocyanin molecules were observed in areas which were sparsely labelled, but this designation indicated that an occasional hemocyanin molecule was viewed.

Preparation of hemocyanin: Hemocyanin was obtained from the Atlantic Whelk (*Busycon canaliculata*) utilizing the procedure of Perkins and Koehler (5). A knife was thrust through the operculum into the heart and the circulatory fluid allowed to drain into a beaker. The fluid was then centrifuged at low speed (5,000 × g) at 4 C to remove debris and the supernatant de-

canted and centrifuged at 18,000 × g at 4 C to concentrate the hemocyanin. Concentrated hemocyanin was resuspended in 3% NaCl, sterilized by filtration (.45 μm filter), and stored at 4 C until used.

RESULTS

The head: The cuticle of the head region of mature *C. elegans* exhibited light to heavy label from the first to the fifth annule (Figs. 1, 2). Posterior to the fifth annule labelling was sparse. Exudates frequently were observed which appeared to have emanated from the amphid or the inner labial papillae (Fig. 3). No labelling was observed on these putative secretions. Occasionally the label adhered to the papillae or the opening of the amphid, but generally the major portion of the label was limited to areas of the annule posterior to these structures.

The cuticle of *M. incognita* also generally labelled heavily from the first to the fifth annule, with a few specimens showing moderate labelling (Fig. 4). The lateral lips, medial lips, and labial discs also labelled heavily. Posterior to the fifth annule labelling was sparse.

Nonspecific binding of hemocyanin did not occur.

Midbody and vulva: Without exception, the midbody of both *C. elegans* and *M. incognita* showed sparse labelling (essentially no labelling). The outer segments of the vulval lips of *C. elegans* also labelled sparsely as did inner portions of the lips of larvae (where they could be seen) and of very young adults. However, in older hermaphrodites the inner portions of the vulval lips often labelled heavily. It was previously shown that the surface of the *C. elegans* eggs contain Con A binding sites (B. Zuckerman, J. Zuckerman, I. Kahane, and S. Himmelhoch, unpublished data), suggesting that the labelling of the inner vulval lips may be attributed to carbohydrate residues which rubbed off the egg during deposition and that the observed labelling did not necessarily indicate that mannoside residues were structural components of the cuticle of the inner vulval lips.

The tail: The tail region of *M. incognita* exhibited light labelling, whereas labelling of the *C. elegans* tail was sparse.

The anus: Labelling on the anus of all *M. incognita* and most *C. elegans* specimens was sparse. However, two *C. elegans* specimens showed very heavy labelling near the cleft of the anus. Because of the infrequency of labelling at this site, we believe that labelling in these two specimens can be attributed to mannoside residues deposited during defecation.

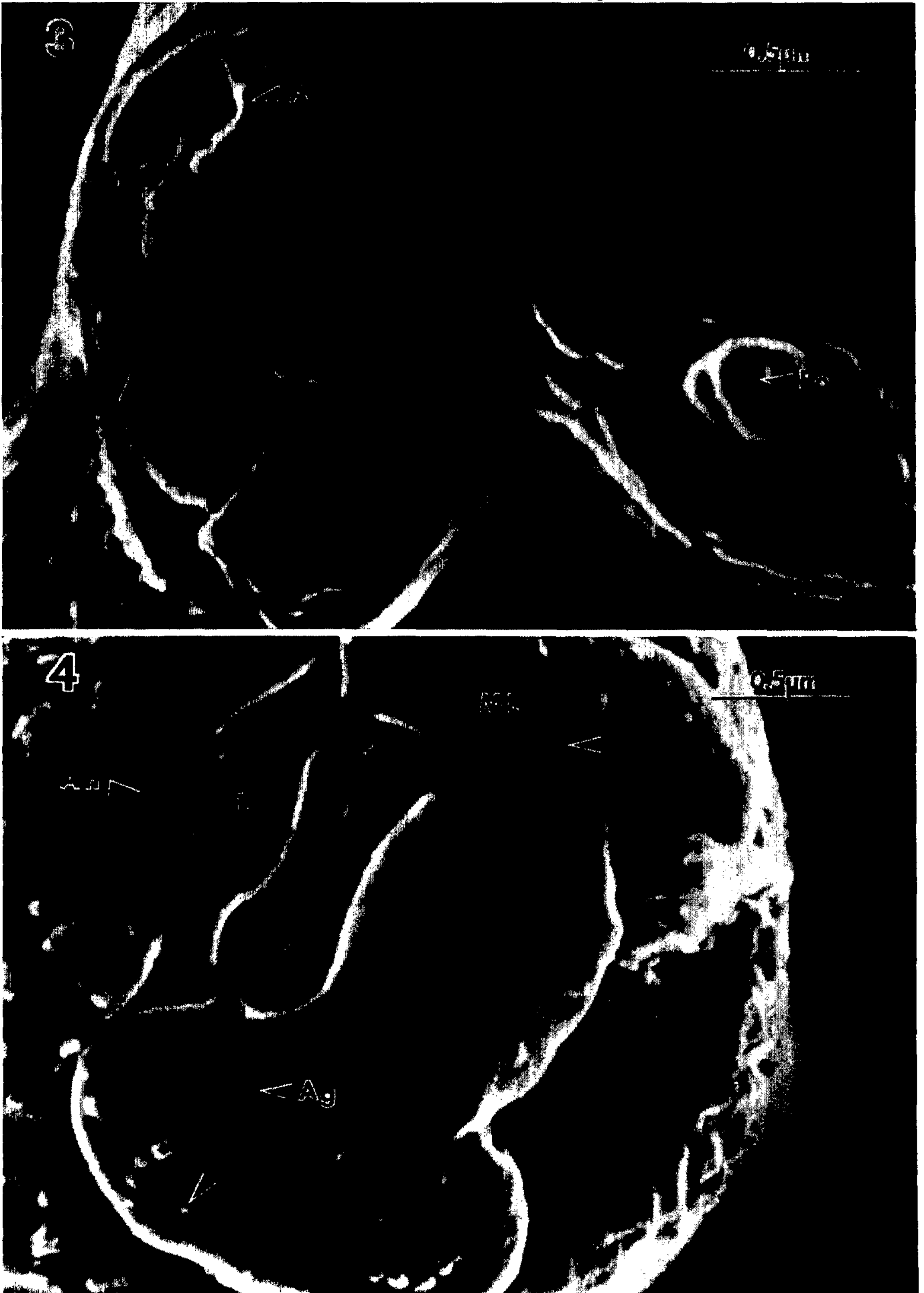
DISCUSSION

The current study demonstrated the clustering and localization of Con A binding sites on the head region of *C. elegans* and *M. incognita* and on the tail of *M. incognita*. Con A binding sites were essentially absent from other areas of the cuticular surface. Where heavy labelling occurred on the inner parts of the vulval lips and the anal region of old *C. elegans*, this labelling probably did not indicate the presence of intrinsic cuticle Con A binding residues for the reasons previously stated. It is premature to speculate on the relation of these findings to nematode behavior, but the possible implications suggest exciting avenues for future research.

The fine structure of the inner labial papillae and amphids of *C. elegans* have been described in detail and this subject has been reviewed by Wright (6). Secretions were frequently observed emanating from the amphids of *M. incognita* and *C. elegans*, and the bleb shown in Figure 3 had every appearance of having been derived from the pore of an inner labial papilla. A similar exudation was observed from many nematodes. Neither the amphidial nor papillar secretions were labelled by the Con A-hemocyanin conjugate, but it is premature to conclude on the basis of the current observations alone that these exudates do not contain Con A specific oligosaccharides.

The current results explain our previous lack of success in consistantly visualizing Con A receptor sites on *C. elegans* by use of a Con A-ferritin conjugate followed by transmission electron microscopy (TEM). In these studies only one of five separate trials showed positive labelling (B. Zuckerman, J. Zuckerman, I. Kahane, and S. Himmelhoch, unpublished data). It is obvious now, since Con A receptors are confined to

Figs. 1–2. Scanning electron micrographs of *Caenorhabditis elegans* treated with Concanavalin A-hemocyanin conjugate. 1) The region of the first five annules (→) which contained Con A binding sites. The hemocyanin molecules cannot be seen at this magnification. Also seen are the papillae (P →) and amphid (A →). 2) Hemocyanin label (→) on cuticle in head region. Several aggregates of hemocyanin molecules can be seen (Ag →).



Figs. 3–4. Scanning electron micrographs of *Caenorhabditis elegans* or *Meloidogyne incognita* treated with Concanavalin A-hemocyanin conjugate. 3) Exudate apparently emanating from the pore of a labial papilla (E →) of *C. elegans.* The pore of another papilla (Po →) and the amphid pore (A →) also are indicated. 4) Hemocyanin label (→) on the lateral lips (L), medial lips (ML), and the first annule (An →) of *M. incognita.* Several aggregates of hemocyanin molecules (Ag →) are visible.

certain areas of the *C. elegans* surface, that most sections viewed by TEM were from unlabelled areas. The hemocyanin labelling technique coupled with SEM has a marked advantage over TEM when it is desirable to localize receptor sites on the surfaces of small metazoa, since the bound hemocyanin molecules can easily be detected by a rapid scan of the whole organism.

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