Fundam. appl. Nematol., 1996, **19** (1), 71-78

Immunocytochemical analysis of the stage-specific distribution of collagen in the cuticle of *Meloidogyne incognita*

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Accepted for publication 11 January 1995.

Summary – Polyclonal antiserum was raised against the major 76 kDa collagen protein extracted from cuticles of *Meloidogyne incognita* adult females. The amino acid composition of this major collagen protein was similar to the amino acid composition of other nematode collagens. In Western blots, the polyclonal antiserum reacted strongly with two collagen proteins (M_r 76 and 140 kDa) in β-mercaptoethanol soluble proteins from cuticles of adult females and with several collagen proteins from preparasitic second-stage juveniles (J2). The polyclonal antiserum was used in immunogold electron microscopy to localize collagen in the cuticles of different life stages and in the egg shell. In vermiform preparasitic J2 and adult males, which have distinctly three-zoned cuticles, dense gold labeling occurred only over the cortical zone and was absent from the epicuticle, the median zone, and striated basal zone. In sausage-shaped parasitic J2, which have a homogeneously structured cuticle, gold particles were uniformly distributed over the entire internal structure of the cuticle. Gold particles also were uniformly distributed on the entire width of the internal structure of the cuticle of the adult female and SDS-purified cuticles from adult females. In the egg shell, gold labeling occurred over the chitin layer.

Résumé – ***Analyse immunocytochimique chez* Meloidogyne incognita *de la répartition du collagène cuticulaire en fonction du stade de développement*** – Il a été produit un antisérum polyclonal dirigé contre la protéine majeure du collagène (76 kDa) extraite de la cuticule de femelles adultes de *Meloidogyne incognita*. La composition en acides aminés de cette protéine est semblable à celle du collagène d'autres nématodes. Parmi les protéines extraites de la cuticule de femelles adultes de *Meloidogyne* et solubles dans le β-mercaptoéthanol, deux protéines du collagène (M_r 76 et 140 kDa) ainsi que plusieurs protéines du collagène de juvéniles de deuxième stade (J2), réagissent au cours d'analyse Western blot avec des anticorps polyclonaux. L'antisérum polyclonal a été utilisé en microscopie électronique pour localiser le collagène dans la cuticule de différents stades et dans la paroi de l'œuf. Chez les J2 libres et les adultes mâles, où la cuticule est formée de trois couches distinctes, un marquage, intense, par l'or ne concerne que la couche corticale, et non l'épicuticule, la couche médiane ou la couche basale striée. Chez les J2 sédentaires enflés, dont la cuticule est de structure homogène, les particules d'or sont réparties dans toute la profondeur de la cuticule. Les particules d'or sont également uniformément réparties dans toute l'épaisseur de la cuticule des femelles adultes, y compris après purification au SDS. Dans la paroi de l'œuf, le marquage à l'or est visible à la surface de la couche chitineuse.

Key-words : collagen, cuticle, immunogold, life stages, plant-parasite, polyclonal antibodies, root-knot nematode.

The nematode cuticle is a physiologically active, extracellular structure that serves as an exoskeleton and as a selectively permeable barrier between the animal and the environment in which it lives. In most nematodes, the cuticle is three-zoned and is divided into an outer cortical zone covered by an epicuticle, a median zone, and an inner basal zone (Bird & Bird, 1991). In contrast to the paucity of data about the biochemical composition of the individual cuticle zones of plant-parasitic nematodes, cuticles of the bacteria-feeding nematodes *Caenorhabditis elegans* (Cox, 1992) and *Panagrellus silusiae* (Leushner *et al.*, 1979), and the animal parasitic *Ascaris* species (Winkfein *et al.*, 1985; Fetterer & Urban, 1988) have been extensively characterized. Collagens are the major structural components of cuticles of these nematode species and are composed of three polypeptide chains each having an average size of *ca* 30 kDa. In *C. elegans*, cuticle collagens are part of a large multigene family which may contain as many as 150 distinct genes (Cox *et al.*, 1984). The polypeptide chains form trimers through triple helices which are extensively cross-linked with covalent tyrosine-tyrosine bonds and interchain disulfide bonds. The latter are reducible by agents such as β-mercaptoethanol (BME) (Cox, 1992; Kramer, 1994). The structure and thickness of cuticles vary among nematode species and among different devel-

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ISSN 1164-5571/96/01 $ 4.00 / © Gauthier-Villars - ORSTOM

opmental stages of the same species, reflecting the various environments in which the nematodes live and develop.

The cuticle of the plant-parasitic nematode *Meloidogyne incognita* (root-knot nematode) like that from other nematodes is composed of several cross-linked collagen proteins (Reddigari *et al.,* 1986). The cuticle of the vermiform preparasitic second-stage juvenile (J2) is similar to the cuticle of the *C. elegans* dauer larva in both its structure and BME solubility (Reddigari *et al.,* 1986). Like the *C. elegans* dauer larva, the *M. incognita* preparasitic J2 is a survival stage, being the only juvenile stage that is free-living in the fluctuating and often adverse soil environment. When the vermiform J2 (15 μm diameter) penetrates a root of a susceptible plant and becomes parasitic, it develops to a sedentary sausage-shaped J2 before molting. The second molt occurs approximately 11-13 days after the initiation of feeding, and development continues until the nematode becomes a saccate (*ca* 400 μm diameter) and sedentary adult female (Triantaphyllou & Hirschmann, 1960). This dramatic change in gross morphology is accompanied by significant biochemical and ultrastructural changes in the cuticle (Reddigari *et al.,* 1986). The cuticle (4-6 μm thick) of the adult female is more susceptible to BME than the cuticle (0.3-0.4 μm thick) of the preparasitic J2 and contains fewer and apparently different collagens than are present in the preparasitic J2 cuticle. Furthermore, the adult female cuticle contains a M_r 76 kDa protein, not present in the preparasitic J2 cuticle, that constitutes greater than 50 % of the total BME-soluble cuticular proteins based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analyses.

In contrast to *M. incognita,* all the other nematode species that have been used for biochemical studies of the cuticle remain vermiform throughout their life cycles. Biochemical changes that occur in the *M. incognita* cuticle during development of vermiform J2 into a saccate adult female could be expected to differ from those occurring in nematode species that do not undergo such developmental changes. Because the presence of the major 76 kDa adult female cuticle collagen (MAFCC) protein correlates with a striking and rare change in nematode morphology, we decided to further characterize this protein and the cuticle of *M. incognita.* This paper reports on the amino acid composition of this major cuticular protein and immunogold localization of collagen in different life stages of *M. incognita* using a polyclonal serum raised against the major collagen protein.

Materials and methods

Nematode culture and isolation

Meloidogyne incognita was propagated on tomato cv. Rutgers in the greenhouse. Vermiform preparasitic J2 and saccate adult females were collected as previously described (Reddigari *et al.,* 1986). Sausage-shaped parasitic J2 were dissected from infected tomato roots 8-10 days after inoculation. Vermiform males were recovered from soil using a Baermann pan. Egg shells were recovered from 25-μm-pore sieves after J2 had hatched.

Cuticle collagen isolation

Collagens were obtained from isolated cuticles using modified procedures of Reddigari *et al.* (1986). Adult female *M. incognita* (0.5 ml) were suspended in 5 ml homogenization buffer [0.05 M Tris-HCl, pH 7.0, 1 mM phenylmethylsulfonyl fluoride (PMSF)] and homogenized by 20 strokes in a Dounce homogenizer. *M. incognita* preparasitic J2 (0.5 ml) were ground under liquid nitrogen in a mortar and pestle before homogenization in the Tris buffer in a ground glass homogenizer. Homogenates were centrifuged 10 min at 2000 *g* and the pellets washed three times with homogenization buffer. The pellets were resuspended in 1 ml STP buffer (0.1 M Tris-HCl, pH 7.0, 1 % SDS, 1 mM PMSF), boiled 2 min, and left overnight at room temperature on a rocking platform. Cuticles were isolated by centrifuging the homogenates for 10 min at 5000 *g* and washing the pellets three times with STP buffer.

BME-soluble proteins were extracted from the cuticles by resuspending the cuticle pellets in 0.5 ml STP containing 5 % BME, heating the samples for 2 min in a boiling water bath, followed by a 8-24 h incubation at room temperature on a rocking platform. After centrifuging for 10 min at 5000 *g,* the supernatants were stored at – 20 °C. Protein concentrations were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard.

Amino acid analysis

BME-soluble proteins from adult female *M. incognita* cuticles were separated by SDS-PAGE on a 7 % gel (Laemmli, 1970). The gel was stained with Coomassie Blue R-250, and the major 76 kDa protein band excised. The gel slice was minced, homogenized, then stirred overnight in 10 mM Tris-HCl, pH 7.0, 5 % BME, 1 % SDS, at 4 °C. Following ultracentrifugation for 30 min at 15 000 rpm in a SW41 rotor, the supernatant was collected and the pellet stirred and centrifuged as above. Supernatants were pooled and dialyzed at 4 °C against several changes of 1 % BME, 5 % acetic acid. Amino acid analysis was done by Genetic Design Inc. (Watertown, MA, USA) using samples that were hydrolyzed for 48 h and processed by standard ion-exchange chromatographic methods.

Antiserum

Polyclonal antiserum was raised against the MAFCC in New Zealand white rabbits. BME-soluble cuticle proteins were separated on SDS-PAGE on 7 % acrylamide gels as described above. The gels were stained with 0.1 % Coomassie Blue R-250 to identify the MAFCC. The MAFCC band was excised, minced into small piec-

es, and emulsified 1:1 (v:v) with complete Freund's adjuvant. One ml was injected subcutaneously into the rabbit. Two similarly prepared booster injections, using incomplete Freund's adjuvant, were administered. Preimmune serum was collected immediately before the first injection and antiserum was collected following the third injection. Antiserum was stored in 1-ml fractions at – 18 °C without preservative.

Immunofluorescence

Immunolocalization of antibody-binding sites was determined by indirect immunofluorescence microscopy of J2 and adult female cuticles. Nematodes were cut and SDS-purified cuticles were prepared as described above and washed in phosphate-buffered saline (PBS) pH 7.4 (137 mM NaCl, 1.4 mM KH_2PO_4, 2.6 mM KCl, 8.1 mM Na_2HPO_4), and blocked in 10 % goat serum (Sigma) in PBS for a minimum of 2 days. Cuticles were further processed for immunofluorescence screening as previously described (Goverse *et al.,* 1994), using the polyclonal antiserum diluted 1:500 in PBS as the primary antibody and fluorescein isothiocyanate (FITC) goat anti-rabbit IgG (Sigma) diluted 1:500 in FITC diluent (10 mM Tris-HCl, pH 7.2, 149 mM NaCl, and 0.02 % sodium azide).

Immunogold electron microscopy

Preparasitic J2, 8 to 10-day-old parasitic J2, adult males, 26 to 32-day-old adult females, SDS-purified adult female cuticles, and empty egg shells were fixed at room temperature in freshly prepared 3 % glutaraldehyde in 0.2 M cacodylate buffer at pH 7.2. Eggs were fixed for 3 h and all other stages were fixed for 2 h, cut in half, and fixed for an additional 2 h. Samples were washed three times in 0.2 M cacodylate buffer and post-fixed in 1 % OsO_4 for 1 h. Samples were washed in cacodylate buffer and dehydrated through a graded 50-100 % series of ethyl alcohol and then infiltrated and embedded in LR White medium-grade resins (Polysciences Inc., Warrington, PA, USA).

Ultrathin (75-80 nm) sections were cut with a diamond knife, collected on Formvar-coated gold slot grids, and processed for on-grid immunogold labelling using the polyclonal antiserum raised against the MAFCC (Hussey *et al.,* 1990). Grids were floated, sections down, on 30- µl drops in polystyrene Petri dishes at room temperature as follows : 1 h on 3 % bovine serum albumin (BSA) in PBS, overnight in a moisture chamber on the MAFCC antiserum diluted 1:5000 with 1 % BSA-PBS, three 10-min washes on 1 % BSA-PBS, 2 h on the secondary antibody goat anti-rabbit IgG coupled with 10 nm colloidal gold (Jansen Life Sciences Products, Piscataway, NJ, USA) diluted 1:100 with 1 % BSA-PBS and centrifuged at low speed, three 5 min PBS washes, and a final wash on deionized water. The immunogold labelled sections were stained with 5 % aqueous uranyl acetate and lead citrate and observed and photographed in a Zeiss EM-10A transmission electron microscope operated at 60 kV. For control sections, the polyclonal antiserum was replaced with preimmune serum or omitted.

Western blotting

Discontinuous denaturing SDS-PAGE was done using 7.5 % Mini-Protean II Ready Gels (Bio-Rad Laboratories, Hercules, CA, USA). For staining, gels were incubated in a solution of 0.1 % Coomassie Brilliant Blue R-250, 40 % methanol, 7.5 % acetic acid followed by destaining in a solution containing 40 % methanol and 10 % acetic acid. Proteins were electrophoretically transferred from the gels to nitrocellulose filters (Towbin *et al.,* 1979) and the filters incubated in PBS containing 5 % nonfat dry milk and 0.02 % sodium azide. The filters were then incubated for 1 h in a primary antibody solution containing the polyclonal antiserum diluted 1:1000 in PBST (PBS containing 0.01 % Tween-20). Following three washes in PBST, the filters were incubated for 1 h in a secondary antibody solution containing alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma Chemical Co., Saint Louis, MO, USA) diluted 1:3000 in PBST. Bound antibody was detected colorimetrically using the substrates 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and *p*-nitro blue tetrazolium chloride (NBT). Control incubations were prepared identically substituting preimmune rabbit serum diluted 1:1000 for the antiserum.

Results

Western blot analysis

The specificity of the polyclonal antiserum was determined by screening Western blots of BME extracts from cuticles of *M. incognita* adult females and preparasitic J2. In addition to recognizing the 76 kDa MAFCC against which the antiserum was raised, antibodies also bound to another band at M_r 140 kDa in the BME extracts of adult female cuticles (Fig. 1). Several proteins were recognized by the antiserum in BME extracts from cuticles of J2 with a major band at approximately 200 kDa (Fig. 1). No binding was observed on identical blots screened with preimmune rabbit serum (not shown).

Amino acid analysis

The amino acid composition of the 76 kDa MAFCC from *M. incognita* adult females has many similarities with the amino acid composition of other nematode collagens. These similarities include large amounts of glycine (23 %), alanine (7 %), proline (9 %), and hydroxyproline (7 %) (Table 1). In addition, the MAFCC contained considerably more acidic than basic amino acids.

Immunofluorescence

The polyclonal antiserum raised against the MAFCC reacted along the cut edges of the SDS-purified cuticles

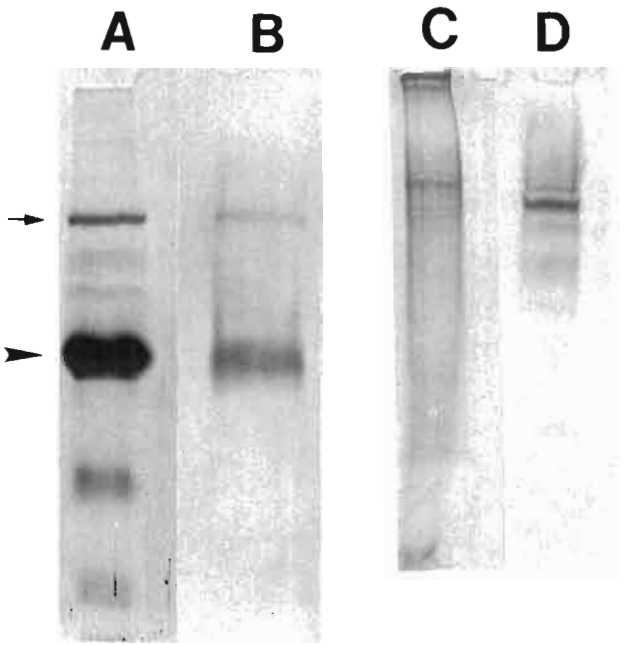

Fig. 1. *Sodium dodecyl sulfate-polyacrylamide gel (7.5 %) electrophoresis of collagen proteins from cuticles of adult female and preparasitic second-stage juvenile* Meloidogyne incognita. *A : Coomassie Blue staining of 20 µg total β-mercaptoethanol-soluble proteins from STP-treated adult female cuticles* (Arrow = 140 000 Dalton protein; arrowhead = 76 000 Dalton protein); *B : Western blot of a similar gel containing 19 ng total adult female cuticle proteins probed with a polyclonal antiserum raised against the 76 kDa protein; C : Coomassie Blue staining of 20 µg total β-mercaptoethanol-soluble proteins from STP-treated juvenile cuticles; D : Western blot of a similar gel containing 75 ng total juvenile cuticle proteins probed with the polyclonal serum.*

Table 1. *Amino acid composition of a major collagen protein isolated from cuticles of adult females of* Meloidogyne incognita. *Residues per 1000 amino acids.*

Amino acid*	Collagen protein
Aspartic acid**	32.73
Threonine	18.22
Serine	51.23
Glutamic acid	109.86
Proline	88.63
Hydroxyproline	68.44
Glycine	227.91
Alanine	71.09
Cysteine	not determined
Valine	16.13
Methionine	0
Isoleucine	19.75
Leucine	14.94
Tyrosine	6.26
Phenylalanine	0
Lysine	23.57
Hydroxylysine	0
Histidine	10.04
Arginine	12.01
Tryptophan	not determined

* Based on 48 h hydrolysis. Analysis was performed by Genetic Design, Inc./Sequemot Inc., Watertown, MA, USA.
** Amidic forms included.

of *M. incognita* J2 and adult females (Fig. 2). No FITC labelling occurred in any cuticles when the preimmune serum was substituted for the primary antiserum (not shown).

Immunogold labelling

Immunogold labelling using the polyclonal antiserum demonstrated the presence of collagen in specific zones of cuticles of different stages of *M. incognita*. In preparasitic J2, dense gold labelling occurred over the cortical zone of the cuticle (Fig. 3 A, B). In longitudinal sections of the cuticle, labelling was absent over the epicuticle, the less structured median zone, and the striated basal zone (Fig. 3 A). In the lateral field of the cuticle, the basal zone is modified and an additional zone occurred between the median and basal zones (Fig. 3 B). In the lateral field, the density of gold particles was greatest over the cortical zone. The invaginated cuticle which lines the nematode's stoma consists of two layers, an electron-transparent inner layer and an electron-opaque outer layer (Fig. 3 C). Dense gold labelling was associated only with the outer layer of the stomatal wall of preparasitic J2.

Following the onset of parasitism and change in body shape, the structure of the J2 cuticle is greatly modified. The characteristic median and striated basal zones present in the cuticle of vermiform preparasitic J2 were no longer evident. In sections of the cuticle of sausage-shaped parasitic J2 gold particles were uniformly distributed over the expanded cortical zone (Fig. 3 D).

The internal structure of the cuticle of the adult female was not clearly separated into distinct zones. This apparent lack of distinct zones was supported by uniform distribution of dense gold particles over the entire internal structure of the cuticle (Fig. 4 A). Similarly, in SDS-purified cuticles from adult females, gold particles were densely deposited over the entire width of the cuticle (Fig. 4 B).

The cuticle of the vermiform adult male of *M. incognita* was three-zoned like the preparasitic J2 cuticle, although the male cuticle was much thicker. In the male cuticle, gold particles were deposited uniformly over the cortical zone, whereas the median and basal zones were unlabeled (Fig. 4 C).

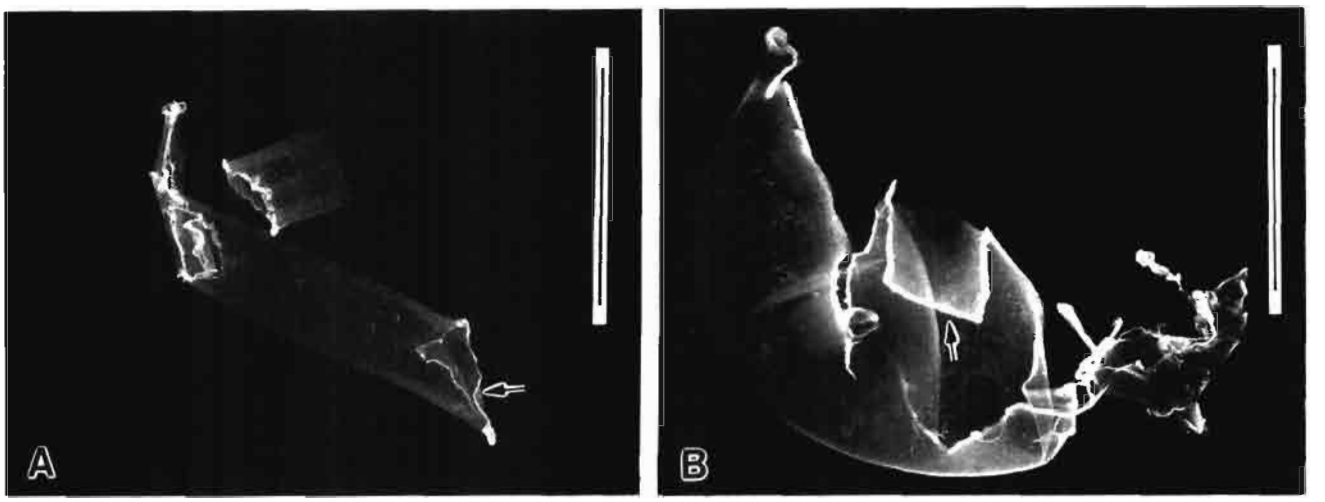

Fig. 2. *Indirect immunofluorescence microscopy demonstrating binding of the polyclonal antiserum used in Fig. 1 to SDS-purified cuticles of* Meloidogyne incognita *second-stage juveniles (A) and adult females (B) (Binding [arrows] of the antiserum is limited to the cut ends of the cuticles; bar = 50 µm in A; 250 µm in B).*

Egg shells consist of an outer vitelline membrane, a thick chitin layer, and an inner lipid layer. Gold particles were deposited over the chitin layer of the egg shell (Fig. 4 D). The pattern of gold deposition over the chitin layer was irregular and usually in aggregates.

No gold labelling occurred in any sections when the primary antiserum was omitted or when preimmune serum was substituted for the primary antiserum (not shown).

Discussion

The polyclonal antibodies raised against the *M. incognita* 76 kDa MAFCC reacted strongly in Western blots with adult female and preparasitic J2 cuticle proteins previously shown to be sensitive to digestion with bacterial collagenase (Reddigari *et al.*, 1986). The antibodies also have been used to isolate from a *M. incognita* adult female cDNA expression library a clone that encodes a collagen protein (Ray & Hussey, unpubl.). Although the polyclonal antibodies were specific for only two collagen proteins present in extracts of cuticles of adult females, the cross reactivity with several collagen proteins in extracts of cuticles of preparasitic J2 was not unexpected. Approximately 50 % of the amino acids of collagen proteins are part of the repeating sequence $(Gly\text{-}X\text{-}Y)_n$ necessary for the polypeptide chains to form the triple-helical conformation characteristic of collagens (Cox, 1992) and, therefore, there may be many common antigenic sites among collagens.

In nematode species from which collagen genes have been isolated, collagen proteins have an average size of *ca* 30 kDa and form multimers of mostly two to four proteins held together by non-reducible bonds (Cox, 1992). The *M. incognita* 76 kDa MAFCC probably represents a dimer of polypeptide chains joined by non-reducible cross links, such as those formed between adjacent tyrosines. The 140 kDa collagen protein that the antiserum also reacted with in adult female cuticle extracts probably represents a tetramer formed by cross-linking of two MAFCC.

The distribution of collagen recognized by the polyclonal antiserum varied in the cuticle zones of the different life stages of *M. incognita*. The greatest variation in collagen distribution occurred in vermiform stages of *M. incognita* that had distinctly layered cuticles. The absence of gold labelling over the median and striated basal zones of the three-zoned cuticles of preparasitic J2 and adult males suggests either that collagen is a minor component of these zones, the epitopes are inaccessible to the antibodies, or possibly these zones are composed of a different collagen species not crossreactive with the antiserum. In previous studies, the striated basal zone of culticles of different nematodes species differed from the other cuticle zones by being the zone least solubilized by reducing agents (Cox *et al.*, 1981; Reddigari *et al.*, 1986). The structural components in the striated basal zone remain unknown. Insoluble cuticle components have been termed cuticlin, but immunocytochemical studies have revealed that cuticlin is present principally in the cortical zone and absent from the striated basal zone of the cuticle of *C. elegans* dauer larvae (Ristoratore *et al.*, 1994). The striated basal zone is considered the most resistant zone of the cuticle and is principally present in life stages requiring the greatest protection from the environment (Bird, 1971).

The cuticle is invaginated at the various openings in the nematode's body and lines the lumen of the oesopha-

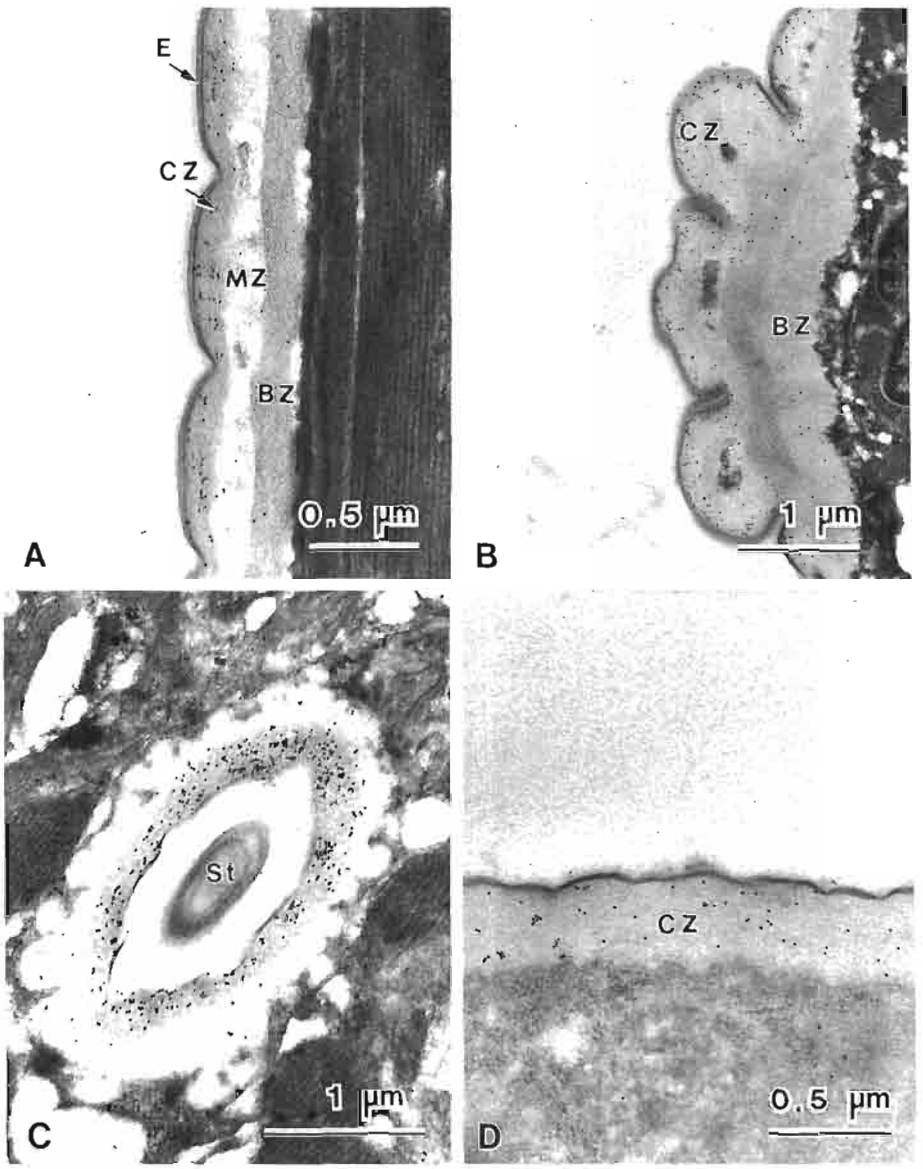

Fig. 3. *Immunocytochemical localization of collagen in cuticles of second-stage juveniles (J2) of* Meloidogyne incognita. *A : Immunogold labelling of collagen in the cortical zone (CZ) of the cuticle of a vermiform preparasitic J2; B : Immunogold labelling of collagen in the CZ in a lateral field of a preparasitic J2; C : Immunogold labelling of collagen in outer layer of the stomatal wall of a preparasitic J2; D : Immunogold labelling of collagen in the cuticle of a sausage-shaped parasitic J2 8-10 day after root penetration.* (E = epicuticle, MZ = median zone, BZ = basal zone, St = stylet).

gus (Bird & Bird, 1991). In ultrastructure studies of *H. glycines*, the cuticle lining the stoma differed structurally from the cuticle covering a nematode's body (Endo, 1983). The association of gold labelling only with the outer layer of the stomatal wall indicates that the layers probably differ in collagen composition.

With the onset of parasitism and several days before molting, the cuticle of *M. incognita* J2 undergoes striking changes in structure, presumably to facilitate the considerable growth and change in body shape that occur in the development of a preparasitic J2 to an adult female. In addition to a great increase in thickness, the principal structural changes in the cuticle are atrophy of the median zone and the striated basal zone (Bird, 1968). The cuticle becomes more homogenous and appears to consist of a single cortical zone. In our study, the existence of a single zone is supported by the uniform distribution of gold labeling over the modified cuticle of the parasitic J2. This structurally modified cuticle persists in the nematode if the J2 develops into an adult female.

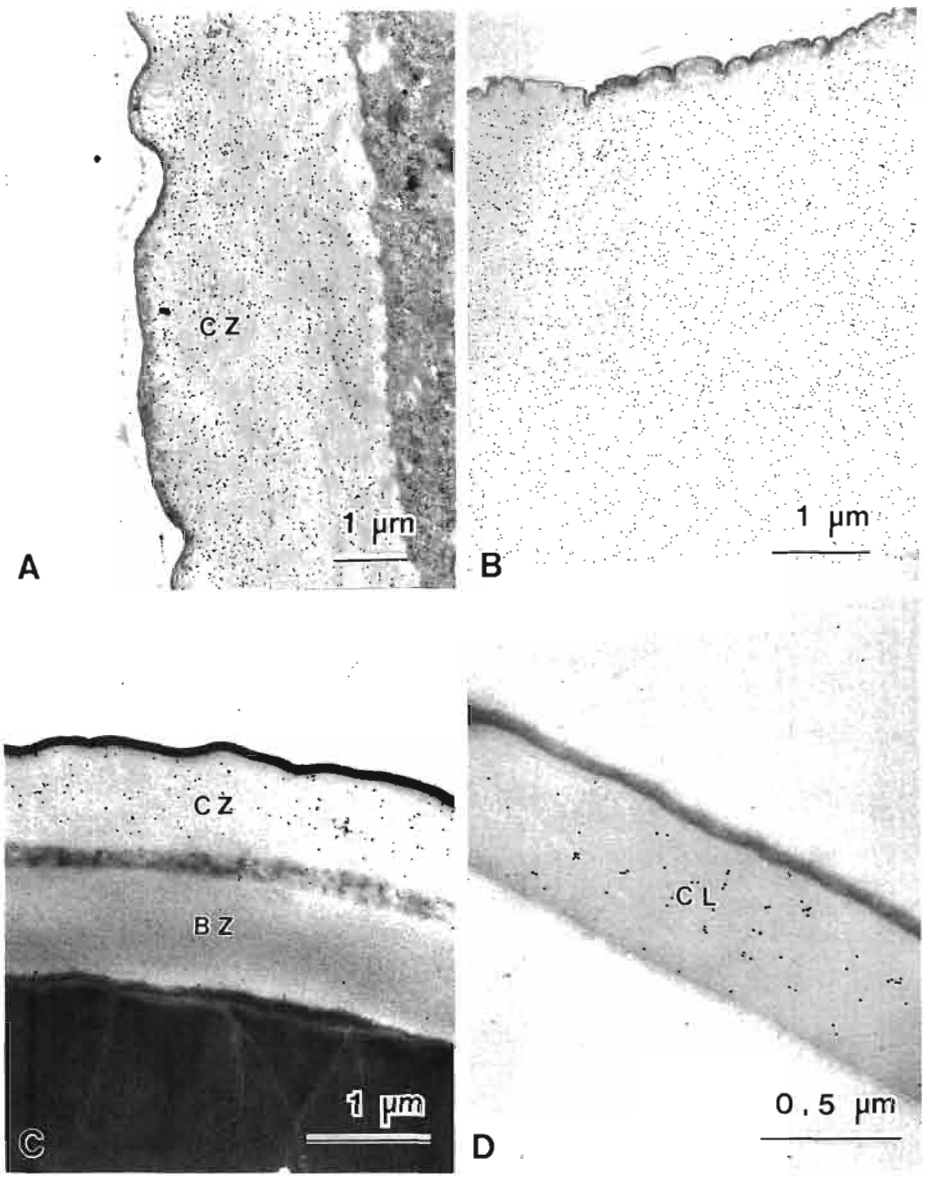

Fig. 4. *Immunocytochemical localization of collagen in cuticles of different life stages of* Meloidogyne incognita. *A : Immunogold labelling of collagen in the cortical zone (CZ) of the cuticle of an adult female; B : Immunogold labelling of collagen in a sodium dodecyl sulfate-purified cuticle from an adult female; C : Immunogold labelling of collagen in the CZ of the cuticle of a vermiform adult male; D : Immunogold labelling of collagen in the chitin layer (CL) of an egg shell.*

The cuticle of the *M. incognita* adult female is much thicker and a fibrous network forms in the cortical zone directly beneath the epicuticle (Reddigari *et al,* 1986). The distribution of gold labelling over the internal structure of the adult female cuticle also suggests a homogenous cuticle consisting of only a cortical zone. However, modification of the adult female cuticle to contain a fiber zone may occur in certain regions of the nematode's body (Bird & Rogers, 1965). The restriction of the immunofluorescence binding pattern of the antiserum to the cut edges of the SDS-purified cuticles is consistent with the distribution of collagen being confined to the internal layers of the cuticle as revealed by immunogold electron microscopy.

The chemical composition of egg shells has been analyzed for several plant-parasitic nematodes (Clarke *et al.,* 1967; Bird & McClure, 1976). Proteins account for greater than 50 % of the composition of the egg shell. Based on the high percentage of proline detected in amino acid analyses, Clarke *et al.* (1967) suggested that

the egg shell contains a collagen-like protein. However, the glycine content (4-10 %) of the egg shell is low for collagens (26 %) (Kramer, 1994). Nevertheless, our immunocytochemical results support the notion that collagen is a structural component of the chitin layer of the egg shell. Furthermore, distribution of the gold labelling suggests that collagen occurs spatially in aggregates in the chitin layer.

In conclusion, striking biochemical and structural changes occur in the cuticle of *M. incognita* as the nematode develops from a mobile, vermiform preparasitic J2 to a sedentary, saccate adult female. The atrophy of the striated basal zone and remodelling of the preparasitic J2 cuticle when the juvenile becomes parasitic is probably required before growth of the nematode's body can occur. In addition, the presence of only a few collagen proteins with molecular weights greater than the 76 kDa MAFCC suggests less cross-linking of collagens in the adult female cuticle.

Acknowledgements

Support for this research was provided by state and Hatch funds allocated to the Georgia Agricultural Experiment Stations.

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