Fundam. appl. Nematol., 1992, **15** (3), 243-250.

Lectin binding to aqueous-soluble and body wall proteins from infective juveniles of *Meloidogyne* species

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Accepted for publication 22 May 1991.

Summary — Nematode surface glycoproteins are important antigens in animal hosts, and they may also play a role in plant parasitism by nematodes. Soluble proteins from homogenates and isolated body wall extracts of *Meloidogyne* spp. second-stage juveniles (J2) demonstrated apparent sugar-specific binding of the biotinylated lectins Concanavalin A (Con A), soybean agglutinin, *Lotus tetragonolobus* agglutinin, and *Limulus polyphemus* agglutinin. The sensitivity of the Western blot assay was enhanced utilizing an alkaline phosphatase-conjugated avidin-biotin complex. Differential protein patterns among *M. incognita, M. javanica,* and *M. arenaria* were detected in all J2 extracts. A high molecular weight protein band difference between host races 1 and 3 of *M. incognita* (Mi1 and Mi3, respectively) was observed in general protein stains of detergent-soluble extracts of isolated J2 body walls. Two Con A-binding protein bands, one greater than 200-kDa and one approximately 100-kDa, were present in Mi1 and absent in Mi3. Binding of Con A to these two protein bands was inhibited in the presence of 100 mM α-methyl mannopyranoside. The potential of glycoproteins in identification and pathogenic specificity among populations of *Meloidogyne* spp. is discussed.

Résumé — ***Lectines s'attachant aux protéines solubles et aux protéines de la paroi du corps des juvéniles infestant de* Meloidogyne *spp*** — Les glycoprotéines de la paroi du corps des nématodes sont des antigènes importants chez certains hôtes animaux; elles pourraient également avoir un rôle dans le parasitisme des nématodes envers les plantes. Des protéines solubles d'homogénats de nématodes et d'extraits de la paroi du corps de juvéniles de deuxième stade (J2) de *Meloidogyne* spp. se sont attachées spécifiquement aux lectines Concanavalin A (Con A) et aux agglutinines de soja, de *Lotus tetragonobolus* et de *Limulus polyphemus.* La sensibilité du test Western blot a été améliorée en utilisant une phosphatase alcaline conjuguée à un complexe avidine-biotine. Des différences ont été notées dans la répartition des protéines des J2 de *M. incognita, M. javanica* et *M. hapla.* Des extraits de protéines de la paroi du corps solubles dans une solution de détergent ont été colorés. Les races 1 et 3 de *M. incognita* (Mi1 et Mi3) ont pu être séparées par des différences dans les protéines de haut poids moléculaire. Deux bandes de protéines s'attachant à Con A — l'une de 100 kDaltons environ, l'autre de plus de 200 kDaltons — sont présentes chez Mi1, mais absentes chez Mi3. L'attachement de Con A à ces deux bandes protéiniques est inhibé en présence de 100 mM de méthyl-mannopyranoside. La possibilité d'utiliser les glycoprotéines pour l'identification des populations de *Meloidogyne* et leur rôle dans la pathogénie spécifique de ces populations sont examinés.

Key-words : Lectins, *Meloidogyne.*

The potential physiological significance of biological molecules that contain carbohydrates (glycoconjugates) in nematode-host recognition and compatibility has recently been postulated (Zuckerman & Jansson, 1984; Kaplan & Davis, 1987). Carbohydrates on nematode surfaces, chemoreceptors, or in secretions may bind to molecular host receptors or external stimuli and invoke a response within the host or nematode. Carbohydrates have been indirectly detected on the surface of root-knot nematodes with fluorescent lectin probes (McClure & Stynes, 1988; Davis *et al.*, 1988; Robertson *et al.*, 1989). Lectins are proteins of nonimmune origin that bind to specific carbohydrates (Goldstein & Poretz, 1986). Treatment of second-stage juveniles (J2) of *Meloidogyne* spp. with various lectins and carbohydrates has led to reduced root penetration and altered tissue response of soybean roots to infection (Davis *et al.*, 1989).

The antigenic determinants of a number of animal parasitic helminths appear to be surface or secretory glycoproteins (Philipp & Rumjaneck, 1984; Almond & Parkhouse, 1985). The removal of antigens from the epicuticle of parasitic nematodes is greatly facilitated by the cationic detergent cetyltrimethylammonium bromide compared to other detergents (Pritchard *et al.*, 1985). Proteins extracted from isolated *M. incognita* cuticles with sodium dodecyl sulfate and β-mercaptoethanol, and a secretory protein from J2 of this nematode stained positive with Periodic-Schiff reagent in SDS-PAGE gels, indicating the presence of carbohy-

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drate components (Reddigari *et al.*, 1986; Hussey *et al.*, 1990).

Probes such as lectins and antibodies will bind to proteins transferred (blotted) to membranes but not to proteins within electrophoretic gels. Our experience indicates that it is difficult to detect small amounts of *Meloidogyne* spp. glycoproteins on western blots using conventional enzyme-conjugated lectins. Probing of western blots with biotinylated lectins (Niedz *et al.*, 1991) combined with the avidin-biotin complex described by Hsu *et al.* (1981) provides an extremely sensitive method to distinguish and characterize glycoproteins separated electrophoretically. This procedure enhances (amplifies) the visibility of lectin binding to nematode glycoprotein bands present in extremely small amounts. Our objectives were to detect and compare glycoproteins from aqueous soluble homogenates and isolated body wall extracts of J2 from several populations of *Meloidogyne* spp. with biotinylated lectin probes.

Materials and methods

Nematode cultures

Populations of *Meloidogyne incognita* races 1 and 3 (Mi1 and Mi3), *M. arenaria* race 1 (Ma), and *M. javanica* (Mj) were maintained in greenhouse culture on roots of tomato (*Lycopersicon esculentum* cv. Rutgers), eggplant (*Solanum melongena* cv. Black Beauty), and pepper (*Capsicum annum* cv. California Wonder). *Meloidogyne* spp. populations were identified by observing adult female perineal patterns, lengths of J2, and development on differential host plants (Sasser & Carter, 1985). Eggs were collected by sieving from host roots massaged by hand in 0.53 % NaOCl for 30 s (Hussey & Barker, 1973). Eggs of each population from combined host roots were hatched on Baermann funnels, each containing gentamicin sulfate (0.1 mg/ml) plus 0.0002 % (w/v) chlorhexidine digluconate to inhibit bacterial and fungal growth. The hatched J2 were transferred to 1.0 ml of sonication buffer (0.05 M Tris-HCl, 1 mM PMSF, pH 7.0) and stored at – 80 °C prior to use.

Chemical abbreviations : Con A, Concanavalin A; SBA, soybean agglutinin; WGA, wheat germ agglutinin; LOT, *Lotus tetragonolobus* agglutinin; LPA, *Limulus polyphemus* agglutinin; BME, β-mercaptoethanol; HEPES, (N-[2-Hydroxyethyl]-piperazine-N′-[2-ethanesulfonic acid]); PMSF, phenylmethylsulfonyl fluoride; CTAB, cetyltrimethylammonium bromide; EDTA, ethylene diaminetetraacetate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; TRIS, tris (hydroxymethyl) aminomethane; BSA, bovine serum albumin; PVDF, polyvinylidenedifluoride; BCIP, 5-bromo-4-chloro-3-indolylphosphate p-toluidine salt; NBT, nitroblue tetrazolium chloride; ABC, avidin-biotin complex.

Protein preparation from J2

Proteins were extracted from stored J2 by sonication (Reddigari *et al.*, 1986; Cox *et al.*, 1981). Aqueous-soluble supernatant from J2 homogenate was collected, clarified by centrifugation, and frozen at – 80 °C prior to use. The presence of isolated body walls in residual pellets of sonicated J2 was confirmed by microscopic observation. Nematode cuticles derived from sonication still have internal muscle proteins attached (Cox *et al.*, 1981), and are referred to here as body walls. Body walls were rinsed five times in sonication buffer and resuspended in 4.0 ml of sonication buffer containing 1.0 % (w/v) SDS or 0.25 % (w/v) CTAB (Pritchard *et al.*, 1985). The SDS suspensions were heated at 100 °C in a boiling water bath for two minutes and incubated overnight at room temperature. The SDS-soluble body wall extract was clarified by centrifugation and collected. The CTAB suspensions were heated in a water bath at 37 °C for four hours, clarified by centrifugation, and the CTAB-soluble extract was collected. All J2 body wall extracts were stored at – 80 °C.

SDS-PAGE and western blots

Extracts from sonicated J2 were thawed, and cuticular proteins were concentrated in sonication buffer by centrifugation in Ultrafree-MC 10 000 NMWL microfuge tubes (Type PLGC, Millipore, Bedford, MA). All protein concentrations were determined according to the method of Lowry *et al.* (1951). Samples from each *Meloidogyne* spp. population were run in discontinuous SDS-PAGE as described by Laemmli (1970). Individual samples of Mi1, Mi3, Mj, and Ma from the same extraction method were run in adjacent lanes to form a " set " for population comparisons, and four identical sets of proteins were included in each electrophoretic run and Western blot. Proteins were separated at a constant 20 mA per gel for 50 min in a 7 cm × 8 cm × 0.75 mm vertical slab gel (4.0 % stacking, 12.0 % separating polyacrylamide gel) with a Mini-Protean II apparatus (Bio-Rad Laboratories, Richmond, CA). Biotinylated SDS-PAGE molecular weight standards (Vector Laboratories, Burlingame, CA) were included in each gel to be electroblotted and probed with biotinylated lectin. High molecular weight SDS-PAGE standards (Bio-Rad Laboratories, Richmond, CA) were included in each gel to be electroblotted and treated with a general protein stain.

Immediately after electrophoresis, gels were equilibrated for 30 min in transfer buffer : Tris/glycine (14 mM/11 mM), 0.0375 % SDS (w/v), 20 % methanol (v/v), pH 8.0. The PVDF transfer membrane (Immobilon-P, Millipore, Bedford, MA) was activated in methanol for 20 s, rinsed three times in distilled water, and equilibrated in transfer buffer for fifteen minutes prior to electroblotting. The gel and membrane were " sandwiched " together and protein bands were electrically transferred from gels to PVDF membranes at a constant

250 mA for one and one-half hours using a Mini-Trans Blot apparatus (Bio-Rad Laboratories, Richmond, CA) filled with cold transfer buffer and continuously cooled with ice. Western blots to be probed with biotinylated lectins were rinsed three times in HEPES buffer saline (HBS : 10 mm HEPES, 150 mM NaCl, 0.1 mM $CaCl_2 \cdot 2H_2O$, 0.1 mM $MgCl_2 \cdot 6H_2O$, 0.01 mM $MnCl_2 \cdot 4\ H_2O$, pH 7.5) and incubated in HBS plus 1.0 % BSA (w/v) at 4 °C overnight to block nonspecific membrane binding sites. This procedure saturates (blocks) portions of the membrane that do not contain protein bands (background) with a protein that will not bind to lectins or avidin-biotin complex. Blots for general protein stain were immersed for 30 min in aqueous 0.1 % (w/v) naphthol blue-black containing 40 % methanol and 10 % glacial acetic acid. General protein patterns were visualized by destaining blots in staining solution minus naphthol blue-black.

LECTIN PROBES OF WESTERN BLOTS

Blots incubated in BSA blocking solution were rinsed three times in HBS. The four sets of J2 proteins on Western blots were divided equally by cutting the transfer membrane and probed as described below. One blot (set) was incubated for 1 h at room temperature in 5.0 ml HBS containing either 2.0 µg/ml biotinylated Con A, SBA, WGA, LOT, or 5.0 µg/ml LPA (E-Y Laboratories, San Mateo, CA). The second blot was incubated for one hour in one of the above lectin solutions plus an appropriate competitive sugar. Each lectin was preincubated for one hour with 100 mM of the appropriate sugar to compete for lectin-sugar binding sites : α-methyl mannopyrannoside for Con A, N-Acetyl-galactosamine for SBA, N-Acetylglucosamine for WGA, α-L-fucose for LOT, and N-Acetylneuraminic (sialic) acid for LPA. Competitive sugars should inhibit binding of lectins to some glycoprotein bands if true lectin-carbohydrate binding has occurred. The remaining two blots were incubated in HBS minus lectin and sugar to serve as control blots for nonspecific binding of the reagents listed below to protein bands.

Alkaline phosphatase-labeled avidin will bind to the biotin attached to lectins that have bound to nematode glycoprotein bands. A colored alkaline phosphatase substrate will then make visible the glycoprotein bands that have bound lectin. One hundred microliters of each reagent, A and B, from the Vectastain ABC (avidin-biotin complex)-alkaline phosphatase kit (Vector Laboratories, Burlingame, CA) were mixed in 20 ml HBS for 30 min, prior to use. Avidin has multiple binding sites for biotin and forms large avidin-biotin-alkaline phosphatase complexes when reagents A and B are mixed. Every avidin that binds to biotinylated lectin has many alkaline phosphatases attached to it and effectively amplifies the color reaction when alkaline phosphatase substrate is added. Blots were rinsed three times in HBS to remove biotinylated lectin not bound to glycoprotein bands. Each blot was then incubated in 5.0 ml ABC solution for one and one-half hours at room temperature. Blots were then rinsed three times in HBS to remove unbound ABC and equilibrated for 15 min in substrate buffer (0.1 M Tris-HCl, 0.1 M NaCl, 50 mM $MgCl_2 \cdot 6\ H_2O$, pH 9.5). Substrate solution containing 1.65 mg NBT and 0.85 mg BCIP (Bethesda Research Laboratories, Gaithersburg, MD) per 10 ml substrate buffer was used to visualize alkaline phosphatase labeled proteins. One control blot received 50 µl of levamisole (Vector Laboratories protocol, Burlingame, CA) in 5.0 ml substrate solution to selectively inhibit all (endogenous nematode) alkaline phosphatases except those supplied in the Vectastain kit. The remaining control blot was used to indicate nonspecific binding of ABC to protein bands in the absence of lectin. All blots were incubated in 5.0 ml substrate solution in the dark until bands developed color or background darkened slightly. The substrate reaction was stopped by immersing blots in solution containing 20 mM Tris-HCl, 5.0 mM EDTA, pH 7.5. Blots were photographed immediately after developing with substrate to record results. The experiments and results reported here were repeated at least once.

Results

General protein staining of SDS-PAGE separations of aqueous soluble extracts from *Meloidogyne* spp. J2 transferred to PVDF membranes revealed up to 21 discrete bands per population from 20 µg of protein per lane. Five protein bands within the range of 50-150 kDa were present in Mi1 and Mi3 but absent from Ma or Mj. A protein band of approximately 50-kDa was present in Ma and Mj and absent from Mi1 and Mi3. A protein band of approximately 80-kDa was present in all populations except Ma, and a protein band of approximately 45-kDa was detected in only Ma.

Species-specific glycoprotein patterns also were detected when Western blots of aqueous-soluble proteins were probed with biotinylated lectins (Table 1). Very weak, nonspecific binding of ABC to aqueous-soluble proteins was observed for eleven bands of protein from Mi1 and Mi3 ranging from approximately 35-kDa to 130-kDa, four bands of protein from Ma ranging from approximately 35-kDa to 40-kDa, and no nonspecific ABC bands from Mj. None of the nonspecific ABC-binding protein bands apparently represented active, endogenous nematode alkaline phosphatases, since no inhibition by levamisole was observed. In general, inhibition of lectin binding by competitive sugars occurred with relatively high molecular weight proteins. Inhibition of lectin binding to protein bands by competitive sugars differed among nematode species.

In aqueous-soluble J2 extracts, as many as twenty protein bands per nematode species bound Con A on Western blots. Competitive sugar inhibition of Con A

Table 1. Approximate molecular weights[a] of aqueous-soluble proteins derived[b] from isolated body walls and homogenates of whole second-stage juveniles (J2) of *Meloidogyne* spp. populations that exhibit differential " sugar-specific " binding[c] of biotinylated lectins among the populations studied.

Lectin	*M. arenaria*	*M. javanica*	*M. incognita* race 3	*M. incognita* race 1
AQUEOUS-SOLUBLE J2 HOMOGENATE PROTEINS				
Con A	–	228	–	–
	–	–	214	214
	132	132	–	–
	74	74	–	–
	40	–	–	–
SBA	75	–	75	75
	64	–	64	64
LOT	132	132	–	–
	–	–	87	87
	55	–	55	55
LPA	120	120	–	–
	90	–	90	90
	68	–	68	68
SDS-SOLUBLE J2 BODY WALL PROTEINS				
Con A	–	–	132*	–
	–	102	102	102
SBA	170*	–	170*	–
	68	–	–	–
LOT	–	–	105	105
	–	68	68	68
CTAB-SOLUBLE J2 BODY WALL PROTEINS				
Con A	248	–	–	–
	222	–	–	222
	120	–	–	–
	110	–	–	110
	–	35	–	–
LOT	–	152	–	–
	132	132	–	–
	–	95	95	95
	68	–	68	68

[a] : Molecular weights (kilodaltons) were estimated from biotinylated molecular weight markers (Vector Laboratories, Burlingame, CA) run concomitantly with protein samples above in 12 % SDS-PAGE and electroblotted onto polyvinylidene difluoride membranes — [b] : Whole nematodes were homogenized by sonication in 0.05 M Tris-HCl, pH 7.0, plus 1 mM phenylmethylsulfonyl fluoride to produce aqueous-soluble proteins and residual body wall pellets. Proteins were extracted from nematode body walls with either 1.0 % SDS or 0.25 % cetyltrimethylammonium bromide (CTAB) detergent in sonication buffer — [c] : Only lectin binding inhibited in the presence of 100 mM competitive-binding sugar is listed above; α-methyl manno-pyranoside for Concanavalin A (Con A), N-acetyl-galactosamine for soybean agglutinin (SBA), α-L-fucose for *Lotus tetragonolobus* agglutinin (LOT), and N-acetyl-neuraminic (sialic) acid for *Limulus polyphemus* agglutinin (LPA). No binding of wheat germ agglutinin to protein bands was inhibited by 100 mM N-acetyl-glucosamine or differential among nematode populations.

* Protein bands labeled with biotinylated lectin were barely visible.

binding included ten bands for Ma and Mj, and eight bands for Mi1 and Mi3. Up to thirteen protein bands per species bound SBA, but SBA binding to only three bands was inhibited by competitive sugar. Twenty-one identical protein bands from each species bound WGA, and no WGA binding was inhibited by competitive sugar. LOT bound to as many as twelve protein bands per nematode species, but inhibition of LOT binding by competitive sugar was observed for only three bands in Ma, Mi3 and Mi1, and two bands in Mj. Binding of LPA to proteins of approximately 120-kDa, 80-kDa, and 70-kDa was inhibited in the presence of N-acetyl-neuraminic acid, indirectly indicating the presence of sialic acid on these proteins (Fig. 1). The aqueous-soluble protein patterns displayed by LPA and LOT that were inhibited by competitive sugar were very similar in appearance.

Several high molecular weight proteins not present in aqueous-soluble J2 homogenates were detected in general protein patterns of SDS-soluble J2 body wall extracts. Protein bands of approximately 230-kDa, 210-kDa, 160-kDa, 140-kDa, 120-kDa, and 75-kDa were detected in all populations except Mj. Protein bands of approximately 60-kDa and 50-kDa were detected in all populations except Ma. Protein bands of approximately 90-kDa and 30-kDa were unique to Mj.

Species-specific patterns of lectin binding were observed for Con A, SBA, and LOT on Western blots of SDS-soluble J2 body wall extracts (Table 1). No binding of LPA to protein bands from any J2 body wall extracts was detected. Nonspecific binding of ABC to protein bands and active endogenous nematode alkaline phosphatases were not detected in any J2 body wall extracts.

Up to thirteen protein bands per nematode species from SDS-soluble J2 body wall extracts bound Con A on Western blots. Competitive sugar inhibition of Con A binding included one band for Ma, two bands for Mj and Mi1, and three bands for Mi3. As many as thirteen bands per species bound SBA, but sugar inhibition of SBA binding was observed for five, three, four, and three protein bands from Ma, Mj, Mi3, and Mi1, respectively. Eleven identical protein bands from each species bound WGA, and no WGA binding was inhibited by competitive sugar. LOT bound to as many as nine protein bands per nematode species, but inhibition of LOT binding by competitive sugar was observed for only one band in Ma, two bands in Mj, and three bands in Mi1 and Mi3.

Apparent species and race-specific general protein patterns were detected in CTAB-soluble body wall extracts from J2 of *Meloidogyne* spp. with as little as 13 µg of protein per lane (Fig. 2). Only Con A, WGA,

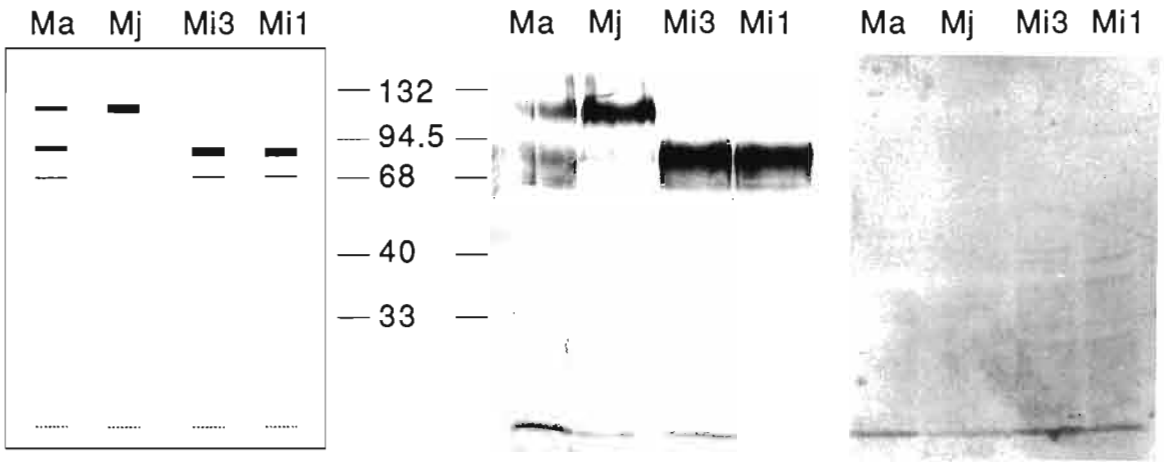

Fig. 1. Line drawing and photographs of Western blots of SDS-PAGE separation of aqueous-soluble homogenates from second-stage juveniles of *Meloidogyne* spp. (20 µg protein/lane) probed with (left and center) biotinylated *Limulus polyphemus* agglutinin and (right) biotinylated *Limulus polyphemus* agglutinin plus 100 mM N-Acetylneuraminic (sialic) acid *M. arenaria* race 1 (Ma); *M. javanica* (Mj); *M. incognita* race 3 (Mi 3); *M. incognita* race 1 (Mi1). Electrophoretic migration of biotinylated molecular weight standards is represented as kilodaltons (kDa).

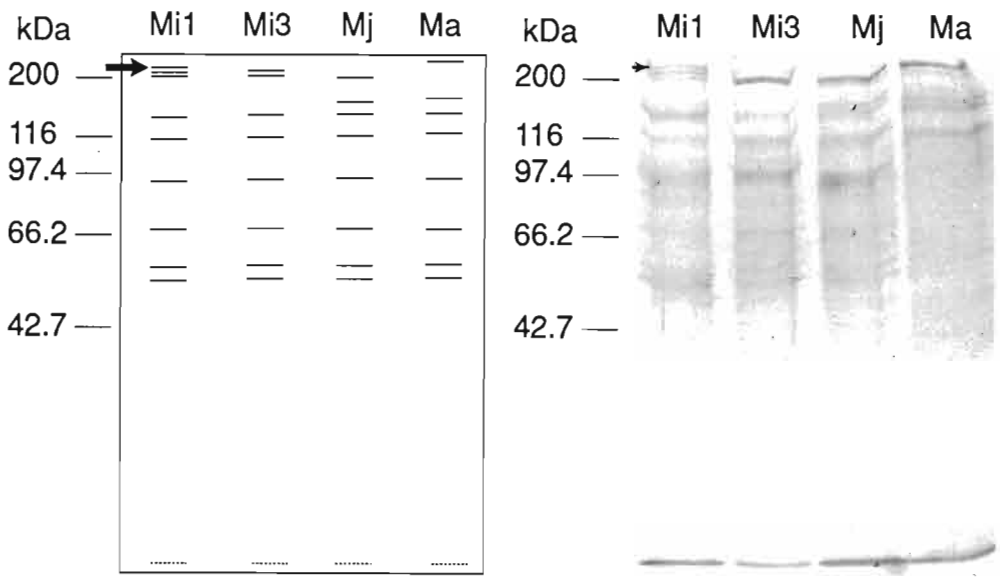

Fig. 2. Line drawing and photograph of SDS-PAGE separation of CTAB-soluble body wall extracts from second-stage juveniles of *Meloidogyne* spp. (13 µg protein/lane) transferred to a PVDF membrane and treated with the general protein stain, naphthol blue-black. *M. incognita* race 1 (Mi1); *M. incognita* race 3 (Mi3); *M. javanica* (Mj); *M. arenaria* race 1 (Ma). Arrow indicates a protein band unique to host race 1 of *M. incognita*. Electrophoretic migration of molecular weight standards is represented as kilodaltons (kDa).

and LOT bound to protein bands from CTAB-soluble J2 body wall extracts (Table 1). Species and race-specific binding of Con A to these CTAB-soluble J2 body wall extracts was obvious, and Con A binding to most of these protein bands was inhibited by competitive sugar (Fig. 3). WGA bound to sixteen identical protein bands from each nematode species, but no WGA binding was inhibited by competitive sugar. LOT bound to as many

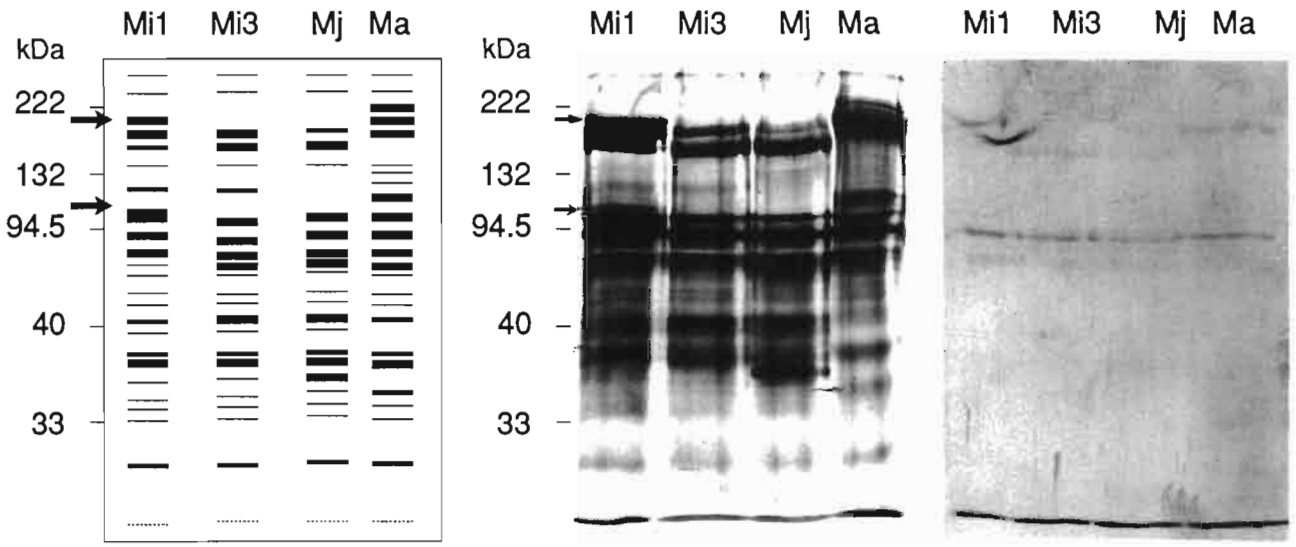

Fig. 3. Line drawing and photographs of Western blots of SDS-PAGE separation of CTAB-soluble body wall extracts from second-stage juveniles of *Meloidogyne* spp. (13 µg protein/lane) probed with (left and center) biotinylated Concanavalin A and (right) biotinylated Concanavalin A plus 100 mM α-methyl mannopyrannoside. *M. incognita* race 1 (Mi1); *M. incognita* race 3 (Mi3); *M. javanica* (Mj); *M. arenaria* race 1 (Ma). Arrows indicate Con A-binding protein bands present in Mi1 but absent from Mi3. Electrophoretic migration of biotinylated molecular weight standards is represented as kilodaltons (kDa).

as four protein bands per species, and LOT binding to all CTAB-soluble bands was inhibited by competitive sugar.

Discussion

A number of glycoproteins from aqueous-soluble homogenates and body wall extracts of *Meloidogyne* spp. J2 were detected, and their carbohydrate residues were partially characterized, by probing of Western blots with biotinylated lectins. Some of these were relatively high molecular weight (> 100-kDa) glycoproteins that were not detected by general protein stains of proteins transferred to PVDF membranes. It is unknown if protease activity that was not inhibited by PMSF affected protein banding patterns observed on PVDF membranes. Repeated gel runs were conducted using aliquots frozen from the same original extracts, and PMSF was the only protease inhibitor utilized. Potential effects of sonication on protein structure are also unknown (Suslick, 1989). General protein patterns differed among aqueous-soluble J2 homogenates and SDS-soluble body wall extracts of *Meloidogyne* species. Differences in general soluble protein patterns among several *Meloidogyne* species have been previously reported (Dickson *et al.*, 1970). To our knowledge, this is the first report of differences in general CTAB-soluble J2 body wall proteins observed between races 1 and 3 of *M. incognita*.

The sensitivity of biotinylated lectin-ABC probing of Western blots was demonstrated by the discrete, intensely-labeled protein bands evident after binding minimal amounts of lectin to minimal amounts of J2 protein. Lectin binding to many of these protein bands was not inhibited by appropriate competitive sugar, indicating potential nonsugar-specific binding of lectin to these protein bands. A number of the glycoproteins detected, however, did exhibit apparent sugar-specific binding of lectins. Competitive sugar assays may also represent the increased or decreased affinity of the lectins for competing haptens *versus* the existing carbohydrate moieties of glycoconjugates (Goldstein & Poretz, 1986). The pH of exogenous sialic acid may affect LPA binding to glycoproteins or LPA may bind to phosphorylcholine (Robey & Liu, 1981). Competitive sugar-lectin assays are encouraging, but only direct chemical analysis can confirm the presence or absence of specific carbohydrates in nematodes. The absence of sialic acids in *Caenorhabditis elegans* and *Panagrellus redivivus* has been determined by direct assay (Basic *et al.*, 1990). Weak, nonspecific binding of ABC to some protein bands in aqueous-soluble J2 homogenates was apparent, but this was easily discernible from nonspecific lectin binding to protein bands. Our inability to detect endogenous alkaline phosphatase in any J2 protein sample is possibly due to the protein denaturation that occurs during SDS-PAGE.

The structural and functional significance of glycoproteins in phytoparasitic nematodes requires elucidation. Glycoproteins may play a role in pathogenicity and host-specificity. A high molecular weight glycoprotein is a component of secretory granules in the eso-

phageal glands of individuals of *M. incognita* (Hussey *et al.*, 1990). This secretory glycoprotein, and other glycoproteins detected here, may function in plant-nematode compatibility (Hussey, 1989). In contrast, it is possible that *Meloidogyne* spp. J2 glycoproteins function as recognition molecules or elicitors of the hypersensitive response in pathogen-induced incompatibility with specific plant cultivars (Kaplan & Davis, 1987; Anderson, 1989). Differences in carbohydrate content of glycoproteins among species, and among races in the case of body wall glycoproteins, may be related to host-specificity. Isolated body walls of *Meloidogyne* spp. J2, and CTAB-soluble J2 body wall extracts in particular, appear to be relatively strong elicitors of phytoalexin accumulation in soybean plant tissue (Davis & Kaplan, 1990) and may be related to soybean incompatibility with root-knot nematodes (Kaplan *et al.*, 1980). Differential glycoconjugates potentially important in these interactions are most likely borne on the outer surface of infective J2, and this is not confirmed by body wall isolation. The possible production or exposure of elicitors from nematode surfaces or secretions once J2 have entered plant root tissue remains unknown.

The differences in glycoprotein patterns among body wall extracts of the species and races of *Meloidogyne* J2 examined here may possibly be useful in molecular identification of nematode populations. Differential general protein patterns and binding of Con A to CTAB-soluble J2 body wall proteins were strongly apparent between races 1 and 3 of *M. incognita*. Differential body wall glycoproteins may demonstrate some potential for the development of an immunoassay to discriminate among races of *Meloidogyne* spp. Clearly, more extensive testing is necessary to correlate these findings with the present species, physiological race, and pathogenicity data available for *Meloidogyne* (Sasser & Carter, 1985).

Acknowledgments

The authors wish to thank Randy Niedz, Delores Jordan, and Carrie Vanderspool for their ideas and technical assistance.

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