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The Surface Coat of Plant-Parasitic Nematodes: Chemical Composition, Origin, and Biological Role—A Review

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Abstract: Chemical composition, origin, and biological role of the surface coat (SC) of plant-parasitic nematodes are described and compared with those of animal-parasitic and free-living nematodes. The SC of the plant-parasitic nematodes is 5–30 nm thick and is characterized by a net negative charge. It consists, at least in part, of glycoproteins and proteins with various molecular weights, depending upon the nematode species. The lability of its components and the binding of human red blood cells to the surface of many tylenchid plant-parasitic nematodes, as well as the binding of several neoglycoproteins to the root-knot nematode *Meloidogyne*, suggest the presence of carbohydrate-recognition-domains for host plants and parasitic or predatory soil microorganisms (*Pasteuria penetrans* and *Dactylaria* spp., for example). These features may also assist in nematode adaptations to soil environments and to plant hosts with defense mechanisms that depend on reactions to nematode surfaces. Surface coat proteins can be species and race specific, a characteristic with promising diagnostic potential.

Key words: biological control agents, carbohydrate-recognition-domain, glycoprotein, lectin, neoglycoprotein, plant-parasitic nematode, protein, recognition, surface coat.

The multilayered nematode cuticle serves as an exoskeleton and as a barrier that limits entry of substances into the body. Much is known about its chemical composition and ultrastructure (4,9,51,60, 63). It is formed from material secreted by the hypodermis (epidermis). Although there is some confusion regarding nomenclature for cuticular layers, most nematologists follow the convention proposed by Wright (60). The hypodermis is separated from the outer layers by a cell membrane. External to the membrane are cortical layers, which are differentiated morphologically and chemically. The epicuticle, external to the cortical layers, may also be differentiated into two or more layers and, in many nematodes, covered externally by a material visualized in electron microscopy as a fuzzy coating (6,28,60) designated 'glycocalyx' by early investigators (6,28). Glycocalyx is a term often used to describe the carbohydrate-rich peripheral zone at the surface of most eukaryotic cells. Wright (60) preferred the term 'surface coat' (SC), which avoids invoking cell membranes because Foley et al. (18) showed that nematode surfaces do not behave like cell membranes. Bird and Zuckerman (6) used the term SC but anticipated that the term 'glycocalyx' might become more appropriate because it emphasizes the carbohydrate-rich nature of this extracellular peripheral material.

Chemical compositions, origins, and biological roles of surface coats have been reviewed for animal parasitic and free-living nematodes (8,26,29). The purpose of this review is to describe surface coats in phytoparasitic nematodes, using findings from recent studies, and compare their features with those of animal parasitic and free-living nematodes.

Chemistry of the Surface Coat

Little is known about the chemistry of the SC of phytonematodes. Several techniques usually required to characterize carbohydrate, lipid, and protein constituents have been used by different investigators (29). These studies have shown that the entire surface, or sometimes only parts of it, contains various carbohydrate residues that readily bind a number of carbohydrate-specific lectins (45,46,51,55,61).

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In contrast, none of the newly hatched first, second, and early third-stage juvenile of *Ascaris suum,* a parasite of vertebrates, bound any of the lectins tested (21). Failure to bind lectins was also observed in the vertebrate parasite *Brugia malayi* (59). With plant-parasitic forms, the occurrence and distribution of several lectin binding sites on outer surfaces seem to depend on the nematode's parasitic habit and age. For example, pre-parasitic second-stage juveniles (J2) of *Anguina tritici,* a seed gall nematode, tend to exhibit more affinity for lectins than do adults (53), whereas greater diversity of lectins tend to be bound to females of the root-inhabiting nematodes, *Meloidogyne incognita* (53) and *M. javanica* (23) than to J2. For root-parasitic nematodes in general, it is not yet possible to relate specific changes in surface glycoconjugates to the development of juveniles into adults, or to the transition from ectoparasitic to sedentary endoparasitic forms (51). The carbohydrates of the SC appear to be glycoprotein complexes in animal-parasitic nematodes (24) and in free-living nematodes, such as *Panagrellus redivivus* (24). The net charge on surfaces of these nematodes is negative (64,65), and cationic detergents have been used by several investigators to remove surface antigens selectively (39). Surface carbohydrates in the plant-parasitic nematodes, *Tylenchulus semipenetrans* (51) and *Anguina* spp. (6,54), are also reported to consist of glycoproteins.

Lipid components of the SC in plant-parasitic nematodes were demonstrated by lipase treatment (51,54) and later by fluorescent probes (44). Pretreatment of *A. tritici* J2 with lipase increased labeling by the wheat germ agglutinin (WGA) lectin, presumably because removal of lipid residues from the outer cuticle provided better access for the conjugated lectin. The same treatment had no observable effect on the fluorescence of *M. incognita* J2 exposed to fluorescent-conjugated lectins. Extraction and thin-layer chromatographic analysis of *Caenorhabditis elegans* revealed the presence of polar, apolar, and complex glycolipids (7). Fluorescent lipid probes that exhibit fluorescence recovery after photobleaching were found to be surface restricted when applied to several animal-parasitic and free-living nematodes (40,42). While the probe AF18 diffused rapidly and virtually to completion on adults of *C. elegans,* the surfaces of infective juveniles of several animal parasites were found to have no affinity for this and other lipid probes that lacked mobility on surfaces of the species tested (40,42). Restricted mobility of surface lipids indicates either the presence of a resistant insoluble protein matrix or perhaps an unusual lipid-containing SC with a composition different from that of free-living nematodes (41).

Several methods have been used to characterize and map SC proteins. Whole nematodes have been labeled with biotin derivatives or radioiodine, and detergent extracts have been electrophoretically analyzed to identify surface-associated proteins from free-living, plant-parasitic, and animal-parasitic species (7,16,27,42). Several specific surface proteins were characterized on *M. incognita* race 1 using [^{125}I]-Bolton Hunter reagent (43), but caution is necessary when interpreting results obtained with the Iodogen or Bolton-Hunter Reagent techniques because iodination is not always restricted to the SC (30). According to Devaney (16), "labeling with [^{125}I] should never be accepted as proof of a surface location, and confirmation of surface exposure by alternative methods is imperative." Few surface proteins were extracted from J2 of *M. incognita* race 3 (27) and *M. javanica* (Spiegel, Kahane and Sharon, unpubl.) using two different kinds of biotin derivatives. Antibodies raised against the principal SC protein from *M. incognita* race 3 differentiated several species and races of *Meloidogyne* (27).

Lectins have not been reported on surfaces of nematodes. But, human red blood cells (HRBC) bound to the surfaces of several plant-parasitic nematode species and preincubation in a series of carbohydrates inhibited that binding, which was Ca^{++} dependent (52,56). Human red blood cells

also adhered to nylon fibers coated with SC extracted from *M. javanica* J2; binding was Ca^{++} dependent and decreased when the nylon fibers were coated with bovine serum albumin or pre-incubated with fucose and mannose (50,52). Gold-conjugated neoglycoproteins, fucosylated-O-BSA, and mannosylated-O-BSA labeled *M. javanica* J2 over the entire body except for the head region, which was similar to the binding pattern observed with HRBC (50,52). These experiments suggest that HRBC adhesion involves carbohydrate moieties of HRBC and corresponding carbohydrate-recognition domains (CRD) on the nematode surface. This was the first report of a surface CRD in the phylum Nematoda (52,56). A classification of animal lectins (17) proposes several groups, one of which is Ca^{++}-dependent (C-type). Several mannose- and fucose-binding proteins belong to this group. Most of the remaining mannose-binding proteins consist of collagen-like sequences with repeated glycine-X-Y triplets in which X is often proline and Y is often 4-hydroxyproline. They also contain hydroxylysine, to which the carbohydrate moiety is attached, and many cysteine residues like other C-type CRD (17). This lectin type was termed 'collectin' (47). Those proteins that have been identified in the nematode cuticle (11,37, 49), therefore, resemble the C-type CRD.

Unlike animal-parasitic nematodes such as *Ascaris lumbricoides* (37), *Brugia malayi, Brugia pahangi* (49), and *Haemonchus contortus* (11), and the free-living nematodes *C. elegans* (10) and *Panagrellus silusiae* (37), in which collagen-like molecules are located mainly in the inner cuticular layers, the plant-parasitic nematode *M. incognita* has collagen-like proteins distributed throughout the entire cuticle (22). If collectins also occur as extracellular materials in the SC, then the noted differences between HRBC binding in the free-living (*P. redivivus*) and tylenchid phytonematodes (52) may result from species-related collectin specificity.

The term 'nematode surface' has been used to describe the target of various probes applied to intact or sectioned nematode cuticles. These probes also may have an affinity for the epicuticle (44). The fluoresceinated probes (mostly lectins) used to label glycoconjugates on most plant-parasitic nematodes bound to the head region, and only a few lectins bound to the surface of the rest of the body in the few species examined (23,45,53). Electron micrographs demonstrated that binding of lectin was restricted mainly to annular grooves of the outer cuticle (54). Periodate oxidation under mild conditions (4 C, in the dark) increased the fluorescence of *Anguina* species subsequently treated with fluorescent lectins (similarly to the lipase treatment noted earlier) and stripped the SC of these species without eliminating the binding of WGA (33). Treatment of *M. incognita* with periodate (or with lipase) did not enhance binding of lectins, suggesting at least three possibilities: 1) sugar residues in *Anguina* are masked by a lipid layer, 2) there are few if any digestible lipids on the surface of *M. incognita,* or 3) binding is not restricted to the SC and extends to the epicuticle.

The Origin of the Surface Coat

The origin of the SC is a matter of controversy. Several workers have postulated that the SC originates from secretions released by excretory-secretory pores. Polyclonal antibodies to J2 from *M. javanica* reacted with a mixture of antigens from the mouth, excretory pore, and, to a lesser extent, the buccal stylet of both larvae and adults (3). Fluoresceinated WGA bound to the excretory pore exudate and to the outer surface of *A. agrostis,* leading Bird et al. (5) to conclude that glycoconjugates exude from the excretory pore and spread over the nematode surface. Further support for this hypothesis is derived from observations of Premachandran et al. (38) that secretions from the excretory pore, amphids, and phasmids of *M. incognita* stained with the protein-specific dye, Brilliant Blue G (Coomassie Blue). Buccal and excretory exudations, as well as the cuticular coating in the region of the excretory

pore of *M. javanica,* also stained with Coomassie Blue (36). McClure and von Mende (32) induced stylet and (or) amphidial secretions in *Aphelenchus avenae, Heterodera schachtii, M. incognita,* and *Xiphinema americanum* by treatment with toluene, xylene, catechol, resorcinol, hydroquinone, guaiacol, caffeic acid, and serotonin. The secretions also stained with Coomassie Blue, an indication of their proteinaceous nature. But it has not been shown that any of these secretions coat the entire nematode surface. Stained proteins were visible only around the excretory pore and (or) heads of the nematodes examined by light microscopy. Bird and Bird (4), Bird et al. (5), and Bird and Zuckerman (6) all suggested that the excretory system in phytonematodes secretes the SC, that the SC consists of carbohydrate and proteins, and that the protein component can be renewed after degradation by proteolytic enzymes. Nelson and Riddle (34) used a laser microbeam to ablate individual cells of the secretory-excretory system in *C. elegans* and concluded that the secretory system produces the SC. Page et al. (36) came to a similar conclusion regarding the origin of the SC of *Toxocara canis.* Thus, as proposed for different nematode species, it seems likely that the SC originates from the secretory system, but the evidence is mostly circumstantial.

Interactions with Host Materials

Host materials may interact with the SC and affect its external exposure and (or) composition. The interaction may occur during pre-parasitic stages, at the onset of infection, or during development in the plant host. The likely contribution of host materials is a necessary consideration when evaluating features of the plant-parasitic nematode SC. Preparasitic juveniles of *H. schachtii* (2) or *M. incognita* (31), freshly hatched from isolated and purified eggs, have had no direct contact with the host and should be relatively free of host saccharides. Females of these sedentary species, and all stages of *Anguina,* are in intimate contact with host tissues and, consequently, have greater opportunity for acquisition of host materials.

Although preparasitic J2 may be free from host residues within the egg, there is evidence to suggest that lectins adhering to the egg shell can bind to emerging J2 (53). Exposure of preparasitic J2 to host root exudates increased the secretion of materials from amphids of *Globodera rostochiensis* (20) and increased the binding of lectins in the head region of *G. rostochiensis* (19). Thus, root exudates can enhance the production or accessibility of some SC components. Cuticular components on the surface of sedentary (parasitic) J2 of *H. schachtii* (even on juveniles with only their head inside the root) (1) are altered significantly when compared with preparasitic J2. The same phenomenon has been observed in sedentary J2 of *G. rostochiensis* (19) but not in J2 of *Meloidogyne* species (Wyss, pers. comm.). The SC in preparasitic J2 is of unknown origin, whereas at least some of the components of the SC in sedentary J2 are thought to be derived from the cuticle itself (1). The SC of pre- and post-parasitic J2, however, may have similar biological roles as recognition domains.

Proudfoot et al. (40) proposed that components of the surface of animal-parasitic nematodes are reorganized during transition from pre- to post-parasitic forms. Moreover, they suggested that the rapidity of events observed, at least with juveniles of *B. pahangi* and *Acanthocheilonema viteae,* points to factors residing within the cuticle itself that modify nematode surface lipids in response to stimuli encountered during infection (41).

The Biological Role of the Surface Coat

Recent evidence implicates involvement of the SC in interactions between nematodes and microorganisms (see below), as well as between parasitic nematodes and their hosts. In animal parasites the SC is

believed to have an important role in adaptation and survival in paratenic hosts because recognition of exposed surface molecules by the host immune system can contribute to development of an effective immune response (26).

Involvement of the SC in relations between phytonematodes and microorganisms: Predatory and endoparasitic nematophagous fungi possess various specialized structures (traps and spores) that are covered with an adhesive material that holds the nematode firmly. The chemistry of this adhesive material is unknown, but the mechanism is thought to be mediated by a lectin (located on the fungal traps or conidia), which binds to specific carbohydrate residues on the nematode surface (24). Involvement of lectin-carbohydrate interactions in prey recognition was reported first by Nordbring-Hertz and Mattiasson (35) and substantiated by Nordbring-Hertz, Jansson, and their colleagues (24): spore attachment decreased following treatments with the appropriate sialic acid-specific hapten or sialidase. On some occasions the surface carbohydrates were dispersed over the entire cuticle, but more often they were specifically localized on either the head or tail, thus providing an explanation for frequent attachment of fungal traps to the cephalic region (24). Zuckerman and Esnard have recently speculated on the role of surface receptors in the parasitism of nematodes by the fungus *Drechmeria coniospora* (62). They proposed that attachment and signalling processes were mediated by outer (carbohydrate) and inner receptors, respectively, located on the nematode surface. By extending their hypothesis, we suggest that the outer receptors constitute what we described as a CRD (52).

Anguina funesta (*Anguina agrostis*), a seed-gall nematode of annual rye grass, is a vector for *Clavibacter toxicus* (*Corynebacterium rathayi*). Livestock grazing on infected rye grass are poisoned by corynetoxins produced by the bacterium. Cells of the bacterium strongly adhere to nematode cuticular surfaces, and the adhesion may be lectin mediated (5). Bird and Zuckerman (6) and Spiegel and Robertson (54) showed that the outer surfaces of dauer larvae of *A. agrostis* and *A. tritici* are degraded by proteolytic enzymes and contain carbohydrate moieties. Bird and Zuckerman (6) found that pretreatment of the nematode with proteolytic enzymes inhibited the attachment of coryneform bacteria to the nematode surface. Proteins appear to play a critical role in adhesion of these bacteria to the nematode. Adhesion was restored 18 hours after proteolytic enzyme treatments, which suggests that the nematode secreted new proteins involved in the adhesion. Electron microscopy and cytochemical studies (33) confirmed the contribution of the SC to the process of adhesion, but it is not clear which components of the SC are involved. Although complete removal of the SC with periodate prevented bacterial adhesion, juveniles that naturally resisted bacterial adhesion did not lack an SC (33). One explanation is that the SC of those individuals to which bacteria do not adhere naturally lacks crucial components that cannot be observed by the transmission electron microscopy technique used.

Pasteuria spp. are obligate parasites of a number of sedentary plant-parasitic nematodes (14). Their development and life cycle are highly synchronized with those of their host (48,57). Host mortality and the ability of *Pasteuria penetrans* to survive in soil under extreme conditions make *P. penetrans* a promising biocontrol candidate for future suppression of plant-parasitic nematodes. The nature of its endospore attachment to the nematode cuticle is not understood. A biochemical interaction between pathogen endospores and nematode cuticular surfaces was assumed by Stirling et al. (58). Studies with lectins led Stirling et al. (58) to conclude that lectins are not involved in the attachment of *P. penetrans* endospores. A different conclusion was reached by Davies and Danks (13), who proposed that noncollagenous domains on nematode surfaces could interact with N-acetylglucosamine moieties on the endospore surface. This possibility

was not confirmed in a recent attempt using a potential antigenic ladder on the nematode surface; a 190 kD glycoprotein present in the cuticle extract was found to be involved in the attachment of *Pasteuria* endospores to the nematode cuticle (12). In other recent studies, it was reported that antibodies to proteins from *P. penetrans* could prevent attachment of endospores to *M. incognita* and that antibody specificity exists among different isolates of *P. penetrans* (15). Differences in adhesiveness of *P. penetrans* to *M. incognita* and *M. javanica* compared with *M. arenaria* (13) may be explained by interspecific differences in the nematode SC.

Involvement of the SC in nematode-plant interactions: Carbohydrate moieties on nematode surface were suggested as specific elicitors, functioning in the so-called 'determinative' phase of nematode-plant interactions (25). More recent results offer direct evidence for lability and replacement of the SC in plant-parasitic nematodes. Second-stage juveniles of *M. incognita* race 3 labeled with radioiodine and incubated in water for 20 hours released [^{125}]I into the water, indicating that SC proteins may be transitory (27). And, preincubation of *M. javanica* J2 with several detergents (cetyltrimethylammonium bromide, n-octyl glucoside, or sodium dodecyl sulphate) temporarily reduced binding intensity of HRBC to the nematode surface; binding was recovered after 24 hours. Also, binding of HRBC, antibodies to SC, lectins and neoglycoproteins to *M. javanica* J2 surfaces was found to be dependent on nematode age and temperature: optimal binding is normally reached at 37 C, while lowering conditions to 4 C inhibits binding (52, Spiegel, Inbar, Kahane and Sharon, unpubl.). Thus, the sloughing-off process is an active, metabolic event, resembling similar events observed with animal-parasitic nematodes (36). This dynamic property of the phytonematode surface could enable the nematode to avoid recognition phenomena that might lead to resistance responses by the plant.

Summary Of Key Issues

A growing body of evidence indicates that the SC plays an important role in the interaction of nematodes with other organisms during the life cycle. The SC of plant-parasitic nematodes is a thin layer that may cover all or only part of the nematode's surface. While its origin is still obscure, there is good evidence that it can be shed and replaced by living nematodes.

Secretions from the excretory system or other secretory organs may contribute to the composition of the SC. It contains proteins, lipids, and carbohydrates as individual components or as glycoprotein, glycolipid, or lipoprotein complexes. Dyes that normally stain proteins do not stain the outer cuticle, and modification of the SC with lipolytic enzymes gives credence to hypotheses that the proteins extracted from isolated cuticles or live nematodes may derive from more complex substances.

The binding of several neoglycoproteins to *M. javanica* and of human red blood cells to the surface of many tylenchid plant-parasitic nematodes suggests the presence of CRDs that may interact with host plants and soil microorganisms (*Pasteuria penetrans* and *Clavibacter* spp., for example). These CRDs and the ability of nematodes to replace the SC could enable nematode adaptations to soil environments and to plant hosts with defense mechanisms that depend on reactions to nematode surfaces. Surface coat proteins can be species and stage-specific (13,15,27, 42), a phenomenon with considerable diagnostic potential.

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