Wheat Germ Agglutinin Binding to the Outer Cuticle of the Plant-parasitic Nematode *Anguina tritici*¹

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Key words: *Anguina tritici*, lectin, wheat germ agglutinin.

Several carbohydrates have been identified on the surface of free-living, plant-parasitic, and animal-parasitic nematodes (1–3,5,7,8). The function of these carbohydrates is uncertain, but they could be involved in the nematode–host recognition process (9). This note reports on the occurrence and identity of carbohydrates or saccharides on the cuticle of *Anguina tritici*, a host-specific parasite that has a life cycle closely linked with that of its cereal hosts (4).

Infective, second-stage *A. tritici* juveniles were extracted from infected, seedlike wheat galls. The J2 were washed thoroughly with distilled water and phosphate-buffered saline (PBS, pH 7.4) and incubated with different lectins conjugated with fluorescein or rhodamine isothiocyanate (FITC, TRITC) as described elsewhere (2,6). The lectins used were Concanavalin A (Con A), *Dolichos biflorus* agglutinin (DBA), *Limax flavus* agglutinin (LFA), *Limulus polyphemus* agglutinin (LPA), soybean agglutinin (SBA), *Ulex europaeus* agglutinin (UEA), and wheat germ agglutinin (WGA). The conjugated lectins were obtained from Bio-Yeda (Rehovot, Israel) or Sigma (Poole, Dorset, U.K.). The specificity of the observed lectin adsorption and the fluorescence microscopy observations was determined as described elsewhere (2,6). Ferritin conjugated to WGA was obtained from Sigma; nematode treatments and preparations for transmission electron microscopy were done as described by Forrest and Robertson (2). Proteolytic digestion of PBS-washed nematodes and lipase pretreatment were accomplished as described previously (6).

Conjugated WGA was the only lectin that produced a strong fluorescence on the outer surface of *A. tritici*. The entire body surface was strongly labeled (Fig. 1), except for the head where the binding was marked only at the tip region. The specificity of this binding was confirmed by testing all the lectins conjugated with fluorescein or rhodamine isothiocyanate (FITC, TRITC) as described elsewhere (2,6). The lectins used were Concanavalin A (Con A), *Dolichos biflorus* agglutinin (DBA), *Limax flavus* agglutinin (LFA), *Limulus polyphemus* agglutinin (LPA), soybean agglutinin (SBA), *Ulex europaeus* agglutinin (UEA), and wheat germ agglutinin (WGA). The conjugated lectins were obtained from

Received for publication 10 August 1987.

¹ Contribution No. 1933-E, 1987 series, from the Agricultural Research Organization (ARO), The Volcani Center, Bet Dagan, Israel. This research was supported by grant No. 1-910-85 from the US-Israel Binational Agricultural Research and Development Fund (BARD) and by the British Royal Society.

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We are grateful to Mrs. Carol Lyon of the SCRI for her technical assistance.
Figs. 1, 2. Anterior end of *Anguina tritici*. 1) Juvenile labeled with WGA–TRITC. Bar represents 50 µm. 2) Reduced labeling of WGA–TRITC-labeled juvenile following incubation in N-acetyl glucosamine oligomers. Same scale as Figure 1.

Figs. 3–5. Electron micrographs of sections through the cuticle of *A. tritici*. Bars represent 500 nm. 3) Labeling in annular grooves with WGA-ferritin (arrows). Section unstained. 4) As Figure 3 but section stained with uranyl acetate and lead citrate to show structure of cuticle. 5) Tip of head showing labeling with WGA-ferritin.
TABLE 1. Binding of wheat germ agglutinin–fluorescein isothiocyanate (WGA–FITC) conjugate to nontreated or pretreated Anguina tritici with different enzymes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Labeling intensity</th>
<th>Labeling intensity</th>
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<tbody>
<tr>
<td></td>
<td>Body wall</td>
<td>Tip of head</td>
</tr>
<tr>
<td>WGA-FITC (control)</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Pronase</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Protease</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Lipase</td>
<td>++++</td>
<td>++++</td>
</tr>
</tbody>
</table>

- = no labeling; +, ++, ++++, ++++ = increased intensities of labeling.

Sugar residues are probably part of the surface glycoprotein, as in Tylenchulus semipenetrans (7). Pretreatment with lipase increased labeling by WGA (Table 1), probably because removal of lipid residues on the outer cuticle provided better access for the conjugated lectin.

Interestingly, a parasite that is specific to certain cereals, including wheat, labeled only with WGA. Whether this apparent specificity between the carbohydrate on the body surface of A. tritici and the lectin in the host is related to recognition of the host by the nematode or to the nematode seeking to avoid recognition by the host is uncertain.

LITERATURE CITED


