The Binding of Host-Selective Toxin Analogs to Mitochondria from Normal and ‘Texas’ Male Sterile Cytoplasm Maize

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ABSTRACT

Tritium-labeled toxin analogs were prepared by reduction with NaB\textsubscript{3}H\textsubscript{4} of either the toxin from Helminthosporium maydis race T or a toxin component from Phyllosticta maydis. These reduced analogs had high radiochemical specific activities, high biological activities, and plant specificities identical to the native toxins. A filtration assay was developed to test the binding of these labeled analogs to isolated mitochondria. Binding was not energy dependent nor was there measurable matrical uptake. The analogs were shown to be lipophilic, a characteristic which gave rise to considerable nondisplaceable binding. No significant differences were observed in the binding characteristics between the mitochondria from normal and male-sterile (Texas) cytoplasm maize. The findings suggest that, at physiologically relevant concentrations, these toxin analogs permeate the membranes of susceptible and resistant mitochondria alike. The lack of demonstrable specific binding does not rule out the involvement of a classical receptor site but does indicate that other kinds of molecular interactions may be involved in the mechanisms for toxicity and specificity.

Phyllosticta maydis Amy and Nelson and Bipolaris (Helminthosporium) maydis (Nisik. and May.) Shoemaker, race T, produce toxins (PM\textsuperscript{-2} and HmT-toxin) which selectively affect Zea mays L. with Texas male-sterile cytoplasm (cms-T) but not fertile (normal, N) cytoplasm (3, 4, 10, 24). These toxins have been characterized and each consists of a family of related compounds, shown in Figure 1 (6, 13, 14). HmT-toxin consists of compounds with a linear \(\beta\)-oxydioxo polyketol structure and chain lengths which vary from C\textsubscript{23} to C\textsubscript{45}. The PM\textsuperscript{-}toxin family of compounds have shorter chain lengths, C\textsubscript{13} to C\textsubscript{35}, and a linear oxy-oxo polyketol structure.

The toxins appear to affect various physiological processes, but their effects on mitochondrial function have been studied extensively and shown to be specific for cms-T-maize mitochondria (6, 18). A number of toxin analogs have been prepared (22, 23) and shown to have high potencies and the same specificity as the native toxins. Methomyl, an insecticide, bears little structural resemblance to these toxins but demonstrates selective toxicity with a much lower potency (1, 12). These toxins, their analogs and methomyl stimulate NADH oxidation but inhibit malate oxidation in isolated cms-T-mitochondria (1, 6, 12, 18). HmT-toxin and methomyl have been shown to cause leakage of NAD\textsuperscript{+} and coenzyme A from mitochondria (1, 9, 17). HmT-toxin also behaves as a Ca\textsuperscript{2+} ionophore in cms-T-mitochondria but not in N-maize-mitochondria (N-mitochondria) or in cms-T-maize derived microsomes (9) and can form cation-selective channels in artificial phospholipid bilayers (8). Thus, cms-T-mitochondria are selectively affected; but the molecular site(s) of action of these toxins has not been established. Consequently, binding studies were undertaken to investigate possible mitochondrial receptors for these toxins.

MATERIALS AND METHODS

Preparation of Reduced Toxin Analogs. Component C of PM-toxin (PM[C]-toxin) was prepared as previously described (6) and HmT-toxin was prepared by the methods of Kono and Daly (13). Either toxin (about 15 mg) was dissolved in a minimum of anhydrous, reagent grade methanol (5–10 ml) and then reduced with sodium borohydride (5–10 mg) at room temperature (25°C). The reaction was stopped by the addition of two to four drops of 1 N HCl. The resulting mixture was fractionated on a Sephadex LH-20 (Pharmacia) column (30 × 1 cm), which was eluted with methanol at a flow rate of 0.6 ml/min. Fractions (2 ml) containing reduced toxin (normally fractions 6–10) were combined, and the volume was reduced below 2 ml by gentle heating under a stream of N\textsubscript{2}. Acetone was added dropwise to the warm, concentrated solution until the solution became turbid. The solution was then stored at −20°C for 24 to 48 h to precipitate the reduced toxin analogs. The precipitated analogs were recovered by centrifugation and then dried under vacuum at room temperature. Stock solutions and dilutions were made in DMSO (Aldrich Chemical Co.) and stored at 4°C.

Preparation of [\textsuperscript{3}H]-Reduced PM(C)-Toxin and [\textsuperscript{3}H]-Reduced HmT-Toxin. Labeled toxin analogs were prepared by reduction of ketone functions with NaB\textsubscript{3}H\textsubscript{4}. PM(C)-toxin and the native HmT-toxin were used as starting materials. Approximately 15 mg of toxin was reduced with 1.0 Ci of [\textsuperscript{U-3}H]NaBH\textsubscript{4} (Amer- sham, 20 Ci/mmole) as described above. After chromatographic fractionation of the reaction mixture, labile tritium was removed by equilibrating the product with 1 to 2 L of methanol or methanol and water (1:1) using 100 ml portions. The exchange solvent was removed by vacuum distillation and assayed. When the activity of the exchange solvent was consistently below 0.1% of the sample's radioactivity, the equilibration procedure was stopped. The precipitates were white and fluffy and the recovery was at least 90% by weight.

The ketone functions of the labeled analogs were fully reduced as determined by the absence of a carbonyl band in their IR spectra and by their chromatographic mobilities. Each of the

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2 Present address: Ecology and Environment Inc., P. O. Box D, Buffalo, NY 14225.

3 Abbreviations: PM, Phyllosticta maydis; cms, cytoplasmic male sterility; N, normal cytoplasm or male fertile; HmT, Helminthosporium maydis race T.
products was analyzed by TLC by applying a known amount of radioactivity to a plate. After development, the plate was exposed to I2-vapors and the stained spot or spots were outlined. The plate was left in the air to dissipate the absorbed I2 and the absorbent corresponding to the spot (3H-reduced PM(C)-toxin) or spots (3H-reduced HmT-toxin) was scraped from the plate and extracted three times with methanol. For both products greater than 90% of the radioactivity applied was recovered in the respective methanol extracts. The specific radioactivity of [8,16,24-3H]-reduced PM(C)-toxin was 8 Ci/mmol and [3H]-reduced HmT-toxin (label was on the methine carbons, refer to Fig. 1) was 3.8 Ci/mmol. The labeled products were stored as DMSO stock solutions in Teflon vials at −80°C.

Figure 1. The chemical structure of the four major components of HmT-toxin and PM-toxin.

Mitochondrial Preparations. Mitochondria were isolated from etiolated seedlings of Zea mays L. W64ATms (cms-T-mitochondria), W64AN (N-mitochondria) or Glycine max L. Merr. (cv Hobbitt) as described by Payne et al. (20). Organellar integrity was measured by Cyt c oxidation (19). Mitochondria produced by these methods were found to be approximately 80% intact. Mitochondria were used if they had respiratory control ratios greater than two and, for cms-T-mitochondria, if respiration state four was stimulated by at least 200% when NADH was the substrate and the native toxin (PM- or HmT-toxin) concentration was 158 nM. Mitochondrial respiration states have been defined (2) as: state one, ADP limiting and low substrate; state two, substrate limiting; state three, respiratory chain limiting; state four, ADP limiting, high substrate; state five, O2 limiting.

Binding Assays. To minimize the adsorption of toxin by glass (20), all labware was treated with a 5 to 10% (w/v) solution of Surfasil (Pierce Chemical Co.) Mitochondria in assay buffer (0.2 M mannitol, 0.02 M TES, 0.002 M KH2PO4, 0.002 M MgCl2·6H2O, and 0.1% [w/v] BSA, adjusted to pH 7.5 with KOH) were incubated with the appropriate 3H-toxin analog at 27°C for 10 min or a specific time. Unless indicated otherwise the concentration of mitochondrial protein was 5 mg/ml. Toxin and analogs were dissolved in DMSO and introduced in the assay medium so that the final concentration of DMSO was below 1% (v/v). The total volume of the assay medium was 0.05 ml. After incubation, the mitochondrial suspension was diluted with 1 ml of assay buffer and filtered through a double layer of glass fiber filters (Whatman GF/F) that had been washed with 1 ml of assay buffer.

After filtration, the mitochondria and filter were washed with 2 ml of cold (4°C) assay buffer. In blank assays (without mitochondria) the filters retained about 2.5% of the radioactivity after washing. Addition of 5 to 10% (w/v) BSA to the assay buffer did not reduce retention of radioactivity, nor did additional washes with assay buffer. Mitochondrial protein retention was complete. After washing, the filters were removed, placed in liquid scintillation vials, and assayed for radioactivity by liquid scintillation counting.

Specific and nonspecific terms commonly used to describe binding of ligands to receptor and acceptor sites, respectively. To avoid possible confusion with specific biological activity, the terms displaceable (specific) and nondisplaceable (nonspecific) will be used here (7). Total binding was measured as outlined above. Nondisplaceable binding was determined by adding the appropriate nonlabeled toxin analog to the incubation mixture simultaneously with the labeled analog. The ratio of nonlabeled to labeled analog was 100 to 1. The amount of retained 3H-toxin analog in this instance was considered to be nondisplaceable binding. Displaceable binding was the difference between total binding and nondisplaceable binding.

Dilution of the assay medium from 0.05 ml to 1 ml created a temporary nonequilibrium state which could have created errors in the assay if the dissociation of the toxin analog from mitochondria was rapid. Therefore, this possibility was tested with both cms-T- and N-mitochondria (Table I). The results indicated that the dilution step did not cause measurable dissociation.

All binding assays were repeated at least twice with a minimum of three, but usually six, replications of each treatment or time point. Replicates did not differ from their respective mean values by more than 10%.

Analytical Procedures. For 3H-assay, a Packard model PL PRAIS liquid scintillation counter was used with 5.0 ml vials and 3A70 scintillation cocktail from Research Products International. All measurements were quench corrected by the channels ratio method. Surface tension measurements of toxins and analogs were made with a du Nouy tensiometer. Partition coefficients for the 3H-toxin analogs were determined by emulsifying an analog between equal volumes of distilled H2O and 1-octanol.

Table 1. Effect of Dilution Before Filtration in the Binding Assay

<table>
<thead>
<tr>
<th>Treatment* Mitochondrial</th>
<th>Binding</th>
<th>Nondisplaceable sourceb</th>
<th>Displaceable pmol toxin/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diluted T</td>
<td>13.9</td>
<td>9.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Undiluted T</td>
<td>13.5</td>
<td>8.9</td>
<td>4.6</td>
</tr>
<tr>
<td>N</td>
<td>14.0</td>
<td>9.5</td>
<td>4.5</td>
</tr>
<tr>
<td>N</td>
<td>14.3</td>
<td>9.0</td>
<td>4.3</td>
</tr>
</tbody>
</table>

* The binding assay was conducted as described in "Materials and Methods" for the diluted treatment. For the undiluted treatment, the mitochondria were filtered without dilution prior to filtration. [3H]-reduced PM(C)-toxin (158 nM) and [3H]-reduced PM(C)-toxin (15.8 μM) were the ligand and displacing ligand, respectively. b T, cms-T-mitochondria; N, N-mitochondria.
for 5 min at 27°C. The emulsion was broken by centrifugation. The separate phases were then assayed for radioactivity by liquid scintillation counting. Protein concentrations were measured by the method of Lowry et al. (16) with BSA as a standard. Mitochondrial oxidation of NADH and malate was determined with an O2 polarograph (20). TLC procedures were described previously (6, 13).

RESULTS

Biological Activity of 3H-Toxin Analogs. Mitochondria from cms-T-maize were sensitive to all toxins and analogs used in these studies. Figure 2 shows the percent stimulation of NADH oxidation under respiration state-four conditions for various concentrations of HmT-toxin, reduced HmT-toxin, [3H]-reduced HmT-toxin, PM(C)-toxin, and [3H]-reduced PM(C)-toxin. The reduced (3H or 1H) toxin analogs were slightly less potent than the native toxins but still active at 2 pmol/mg protein (5.0 nM). All compounds exhibited specific biological activity, since no effect was observed on N-mitochondria when the analogs were used at concentrations up to 1.6 μM.

Effect of Mitochondrial Function on Binding. The binding of [3H]-reduced PM(C)-toxin to mitochondria in several metabolic states was determined, and it was found that binding was affected neither by the respiration state nor by the structural integrity of the mitochondria (Table II). To assure adequate levels of oxygen, samples were shaken at least once per minute during the incubation period and, when needed, sufficient NADH or ADP was added to maintain a particular respiration state for a minimum of 10 min. Some variation in binding was observed between mitochondrial preparations (Table II). Variation has also been observed in the biological responsiveness to these toxins between cms-T-mitochondrial preparations (data not shown). Therefore, the difference in binding between state three and states one and four, if significant, was not considered great enough to be bio-

![Graph](https://via.placeholder.com/150)

![Graph](https://via.placeholder.com/150)

Fig. 2. Comparison of the biological activity of native toxins and toxin analogs on cms-T-mitochondria. Measurements were made with an O2 polarograph. Results are expressed as percent stimulation of the state four respiration rate of exogenous NADH. Data are shown in the top panel for native HmT-toxin (HmT), reduced HmT-toxin (RT), and [3H]-reduced HmT-toxin (3HRT) and in the bottom panel for native PM(C)-toxin, reduced PM(C)-toxin and [3H]-reduced PM(C)-toxin.

Table II. Binding of [3H]-Reduced PM(C)-Toxin to Mitochondria in Various Metabolic States

<table>
<thead>
<tr>
<th>Respiration State</th>
<th>Mitochondrial Source</th>
<th>Binding&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total</th>
<th>Non-displaceable</th>
<th>Displaceable</th>
<th>pmol toxin/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State 1</td>
<td>T</td>
<td>13.2</td>
<td>9.1</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State 1</td>
<td>N</td>
<td>13.6</td>
<td>8.7</td>
<td>4.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State 1</td>
<td>T</td>
<td>13.8</td>
<td>9.1</td>
<td>4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State 3</td>
<td>T</td>
<td>13.5</td>
<td>9.7</td>
<td>3.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State 4</td>
<td>T</td>
<td>14.2</td>
<td>9.4</td>
<td>4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State 4</td>
<td>N</td>
<td>13.6</td>
<td>8.6</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State 1 (Intact)</td>
<td>T</td>
<td>15.0</td>
<td>8.5</td>
<td>6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State 1 (Intact)</td>
<td>N</td>
<td>14.7</td>
<td>8.5</td>
<td>6.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State 1 (Disrupted)</td>
<td>T</td>
<td>13.8</td>
<td>7.5</td>
<td>6.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State 1 (Disrupted)</td>
<td>N</td>
<td>12.4</td>
<td>5.4</td>
<td>7.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>The binding assays were conducted as described in "Materials and Methods" using [3H]-reduced PM(C)-toxin (158 nM) and [3H]-reduced PM(C)-toxin (15.8 μM) as the ligand and displacing ligand, respectively. Disrupted mitochondria were prepared by osmotic shock. The last four assays were conducted with mitochondrial preparations different than those for the first six assays.  

bT, cms-T-mitochondria; N, N-mitochondria.

chemically important.

The disrupted mitochondria were prepared by suspending mitochondria in an assay buffer that did not contain 0.2 M mannitol as an osmoticum. These mitochondria exhibited obligatory respiration with NADH but did not respond to additions of ADP or native HmT- or PM-toxins. As determined by the Cyt c oxidation assay, the mitochondria were not intact. The lack of difference between binding to disrupted and intact mitochondria indicated that the toxin analog had not accumulated in the matrix. These results also indicated that there were no differences in the binding of [3H]-reduced PM(C)-toxin by cms-T- and N-mitochondria.

Two independent reports indicate that the sensitivity of isolated cms-T-mitochondria to HmT-toxin, and presumably PM-toxin, decays over time. Payne et al. (20) reported a decrease of over 80% in the observed sensitivity to toxin as measured by the percent increase in the state four respiration rate with NADH as the substrate. The decrease was observed over a 20 min period. Pham and Gregory (21) reported a similar decrease in sensitivity. However, their observations indicated that the loss occurred over a period of 6 h. A loss of mitochondrial sensitivity to the toxins might be due to lability of a toxin receptor site. To test this possibility, assay incubation times were varied from 10 to 180 min with no significant difference in binding observed. In another experiment mitochondria were aged over several hours prior to being tested for toxin binding. Freshly prepared mitochondria from both cms-T- and N-maize had respiratory control ratios of about 2.5 with NADH as the substrate. The state four respiratory rate of cms-T-maize mitochondria was stimulated by 175% with reduced PM(C)-toxin at a concentration of 158 nM. After aging the mitochondria on ice for 8 h, the respiratory control ratio dropped to 1.25 for both types of mitochondria, and the response of cms-T-maize mitochondria to toxin was minimal (25% stimulation). However, the displaceable binding did not change over the same time period (3 pmol toxin/mg protein, measured at a labeled toxin analog concentration of 79 nM).

Rates of Association and Dissociation. The rate for association of [3H]-reduced PM(C)-toxin to mitochondria was measured in a large scale assay. At specified time intervals, 0.05 ml aliquots were withdrawn, diluted to 1 ml with assay buffer, and filtered.
After 10 min, total, displaceable, and nondisplaceable binding was near equilibrium for both cms-T- and N-mitochondria (Fig. 3, A and B). The initial rates of binding appeared to be fairly consistent when additions of [3H]-reduced PM(C)-toxin were made, and equilibrium was reestablished after about 10 min (Fig. 3C).

The rate of dissociation was measured in a large scale competition assay. The test solution was divided in half after equilibrium had been reached. To one-half of the mixture [3H]-reduced PM(C)-toxin was added so that the concentration of the unlabeled toxin analog was 100 times greater than the labeled toxin analog. Dissociation occurred at about the same rate as association and a large nondisplaceable binding component remained (Fig. 3D).

Effect of Protein and Ligand Concentrations. Figure 4 shows the extent of binding of [3H]-reduced PM(C)-toxin to various quantities of cms-T- and N-mitochondria at two concentrations of the toxin analog. Below 0.5 mg protein/ml the assay did not appear sensitive enough to detect differences in displaceable binding, if such differences did exist. Larger quantities of mitochondria increased the amount of toxin analog bound so that, at 5.0 mg protein/ml, approximately 40% of the toxin was bound by the mitochondria.

The concentration of [3H]-reduced PM(C)-toxin was varied in assays that contained 5.0 mg protein/ml (Fig. 5). Total binding and nondisplaceable binding increased linearly. It was not possible to test beyond a toxin analog concentration of 790 nm because the toxin became insoluble. Furthermore, because displaceable binding was determined by difference, statistically significant effects could not be measured above 320 to 470 nm.

The high level of nondisplaceable binding suggested that the toxin analog might have partitioned into mitochondrial membranes. Therefore, the partition coefficients for reduced PM(C)-toxin and reduced HmT-toxin were determined with a 1-octanol:water system. The partition coefficients were about 17.8 for reduced PM(C)-toxin and 4.5 for reduced HmT-toxin. Thus, both are lipophilic, but reduced HmT-toxin is somewhat more polar.

To determine if the toxin analogs had surfactant properties, surface tension of toxin solutions was measured. Both analogs caused a decrease of about 12% in the surface tension of water when present at a concentration of 0.01 mg/ml. Thus, they do not appear to act as surfactants.

The conclusion from these experiments was that the nondisplaceable binding was probably due to the relative solubilities of the toxin analogs between the membrane and the bulk solution.
For lipophilic ligands, inclusion of organic solvents in the assay medium has been used to reduce nondisplaceable binding (11). The assay buffer was modified by the addition of 12% (v/v) DMSO. Although the mitochondria exhibited lower respiratory rates and control ratios when in this medium, they remained sensitive to toxin and specificity was unchanged. Experiments were performed similar to those shown in Figure 5 except for the inclusion of DMSO and the use of higher toxin analog concentrations (Fig. 6). An increase in total binding and a marked decrease in nondisplaceable binding resulted in a linear increase in displaceable binding. This result does not seem to indicate the existence of a specific toxin receptor in cmst-toxin.

For comparative purposes the binding of [3H]-reduced HmT-toxin to cmst-toxin was determined. The results shown in Table III indicate that this toxin analog has similar binding properties to [3H]-reduced PM(C)-toxin.

**Binding and Host Specificity.** Oxidation of NADH in cmst-mitochondria is affected by both HmT- and PM-toxins but is not affected in N-mitochondria. However, in our experiments, no differences in the binding behavior of toxin analogs to the two types of mitochondria were apparent, although the labeled analogs exhibited high specific toxicity towards cmst-mitochondria. The binding of [3H]-reduced PM(C)-toxin by soybean mitochondria was also measured. Although somewhat less toxin was bound, the patterns of binding by soybean mitochondria were very similar to maize mitochondria.

**DISCUSSION**

The lack of specificity in the binding of toxin analogs to mitochondria from cmst- and N-maize indicates that either a specific binding receptor does not exist for HmT-toxin or that the methods lacked the sensitivity to detect such binding. For both types of mitochondria, nondisplaceable binding was high, presumably because toxin permeates or adsorbs to the membranes of both cmst- and N-mitochondria. Consequently, a displaceable binding component that was a much smaller component than the nondisplaceable component could have escaped detection.

Most assays were conducted with metabolically active mitochondria, but assays comparing intact and disrupted mitochondria revealed no significant differences. Thus, toxin uptake by the mitochondrial matrix was not included as a total binding component.

Toxin concentration ranges appropriate for bioassays were used in the binding studies and a comparison is in order. A concentration of 158 mM toxin caused a several-fold stimulation of NADH oxidation in cmst-mitochondria (Fig. 2). The toxin to protein ratio in the bioassay was 1.58 nM/mg. In the binding assay a toxin concentration of 158 mM yielded a toxin to protein ratio of 32 nM/mg. Consequently, if a small number of specific receptors were present in cmst-mitochondria, the lower toxin to protein ratio should have enhanced detection.

Displaceable binding became unmeasurable at increasing toxin analog concentrations. This was caused by two problems. A small error in the measurement of large values for either total or nondisplaceable binding would have led to a larger error in the calculated value of displaceable binding. A high concentration of a toxin analog, labeled and unlabeled, in the bulk phase would have increased the partitioning of the toxin into the membrane; therefore, nondisplaceable binding would increase with a concomitant decrease in displaceable binding. The addition of DMSO, which increased the solvating ability of the bulk phase, solved the latter problem. The results of experiments in which DMSO was used demonstrated that the displaceable binding was not saturable. This and the lack of displaceable binding at low protein concentrations and biologically effective toxin concentrations (Fig. 4) do not indicate the existence of a toxin receptor site(s) with high affinity and finite concentration. It must be remembered that displaceable binding is not necessarily equivalent to the binding of a ligand by a receptor (7, 15).

A number of HmT- and PM-toxin analogs have been synthesized and most show high biological activities (23). Suzuki et al. (22) synthesized the B-component of PM-toxin by nonstereospecific methods and showed that it was equally as active and specific in its action as the native toxin component. The native toxins have several chiral centers (14), but the activities of synthetic compounds appear to depend upon the location of functional groups, not their configurations. The high potency of the reduced toxin analogs also indicates that the reduction of ketones to hydroxyl groups has little effect on biological activity. Consequently, if a molecular receptor exists it apparently is not specific for certain functional groups nor for certain stereochem-

![Fig. 6. Binding of [3H]-reduced PM(C)-toxin to cmst- and N-mitochondria, in the presence of 12% DMSO, as a function of labeled toxin analog concentration. Other conditions were as described in Figure 5.](image_url)

**Table III. Binding of [3H]-Reduced HmT-Toxin to cmst-Maize Mitochondria**

<table>
<thead>
<tr>
<th>Toxin Concentration (nM)</th>
<th>Binding*</th>
<th>Total</th>
<th>Non-displaceable</th>
<th>Displaceable</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td>0.0</td>
</tr>
<tr>
<td>128</td>
<td></td>
<td>3.9</td>
<td>1.3</td>
<td>2.6</td>
</tr>
<tr>
<td>256</td>
<td></td>
<td>8.9</td>
<td>2.3</td>
<td>6.6</td>
</tr>
<tr>
<td>384</td>
<td></td>
<td>14.6</td>
<td>2.6</td>
<td>12.0</td>
</tr>
<tr>
<td>512</td>
<td></td>
<td>10.2</td>
<td>3.7</td>
<td>6.5</td>
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</tbody>
</table>

*The binding assays were conducted as described in "Materials and Methods" using [3H]-reduced HmT-toxin (38 nM), and [3H]-reduced HmT-toxin (38 nM) as the ligand and the displacing ligand, respectively.
HmT-toxin has been shown to act as an ionophore, selective for cms-T-mitochondria, and disruption of ionic gradients could account for HmT-toxin's activity on mitochondria (1, 5, 9, 12). Our results do not disagree with this type of action. However, biological specificity cannot be explained by a simple ionophoric mode of action, since HmT-toxin can act as an ionophore in artificial membrane bilayers (12). Holden et al. (8) suggested that cms-T-mitochondria might possess uniquely functioning membrane components which allow the toxin to partition more readily and form ion channels. Our results clearly show no differences of toxin binding or partitioning between cms-T- and N-mitochondria, making this hypothesis untenable. Instead, there may be interactions between toxin molecules and a membrane component(s) of normal mitochondria that prevent the toxin from forming an ion channel, whereas cms-T-mitochondria lack such a component(s).

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LITERATURE CITED