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Letter to the Editor

On the Reported Channel-Forming Activity of HmT Toxin

HmT toxin, produced by the fungus *Helminthosporium maydis*, Race T, uncouples oxidative phosphorylation [1], inhibits malate-dependent electron transport [1], and dissipates membrane potential [5] and calcium gradients [4] in susceptible (cms T) corn mitochondria in nanomolar concentrations. These actions are consistent with those of a toxin which increases membrane permeability to ions. We reported observations of channel behavior when purified HmT toxin was added to the aqueous phase bathing a planar phospholipid bilayer membrane under voltage-clamp conditions [6]. Channels were consistently recorded at concentrations (1 ng/ml in the aqueous phase) that are active on isolated mitochondria. These channels were well behaved: reasonably uniform conductance, ideal selectivity for cations over anions, and conductance directly proportional to the toxin concentration added to the system (over three orders of magnitude). A synthetic toxin analog also demonstrated channel behavior.

While channel formation in a model membrane system was consistent with the biological action of the toxin, we were concerned that the channel activity was a contaminant that co-purified with the native toxin. Finding channel behavior in a synthetic toxin analog preparation was the strongest evidence that convinced us that the channel behavior we observed could be a property of the toxin.

Recently we again questioned the relationship of channel formation to biological activity by attempting to eliminate or separate these phenomena. Jones, Hayon and Busath [7] reported that exposure of gramicidin to UV radiation eliminated gramidicin-induced conductances in membranes due to UV photolysis of tryptophan residues. We reasoned that the polyketol structure of the toxin [8, 9] was unlikely to be effected by UV treatment and thus if channel formation was due to the toxin, both channel formation and biological activity should remain after UV treatment. We subjected both HmT toxin and gramicidin to UV exposure. The ability of both HmT toxin and gramicidin to induce permeability increases in membranes decreased as a function of the length of time of UV exposure (Fig.). The average conductance induced in a membrane by HmT toxin exposed to UV for 60 min was equivalent to two channels (or 1% of the conductance induced by the unexposed toxin).

UV-irradiated HmT toxin (60 min exposure) retained its biological activity while losing the channel behavior. This was ascertained by measuring the effect of HmT toxin on uptake of calcium into susceptible corn mitochondria (Table). Treated and untreated HmT toxin (10 ng/ml) were equally effective in eliminating calcium uptake. The data suggest that the reported channels [6] were not a property of the toxin and were likely due to a peptide contaminant.

Recently we have assayed a number of toxin samples (sent directly from the laboratory of Dr. J.M. Daly) of purified HmT toxin from several independent isolations plus synthetic HmT toxin analogs. We were unable to demonstrate any well-behaved conductances after multiple attempts using different membranes and high concentrations in some cases (*data not shown*). We observed some erratic (noisy) conductances which were not dose dependent and not resolvable into discrete unit conductances (single channels). The odd single channel was observed, but very infrequently. We were able to demonstrate channels with our original toxin preparations on the same membranes, thereby eliminating the membrane as a source of inconsistency. We con-

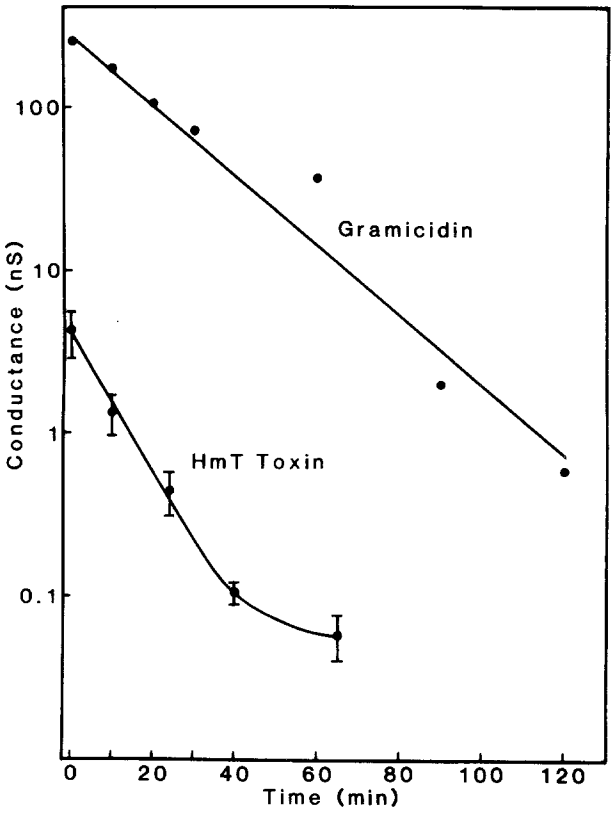

Fig. Gramicidin and HmT toxin channel inactivation by UV irradiation. Gramicidin (50 ng/ml in DMSO) and HmT toxin (5 μg/ml) in quartz cuvettes were exposed to UV irradiation (unfiltered 150 W Xenon arc lamp). Aliquots were removed at intervals and assayed by measurement of the conductance increase induced by HmT toxin or gramicidin in a diphytanoylphosphatidyl choline bilayer (as in ref. 6). HmT toxin (10 μl) was added to an aqueous phase (1 M KCl, 5 mM $CaCl_2$), and the increasing conductance of the bilayer was monitored for 30 min after toxin addition, by which time the conductance had stabilized. The values represent the average of 3–4 separate assays ± SE. Gramicidin (5 μl) was assayed similarly except the aqueous phase was 0.1 M KCl, 5 mM $CaCl_2$. Values are the average of two assays for each time point on separate membranes

Table. Calcium uptake into toxin-susceptible corn mitochondria

	nmol Ca^{2+}/mg prot
Mitochondria	918
Mitochondria + HmT toxin	18
Mitochondria + UV-exposed HmT toxin	23

Malate-dependent $^{45}Ca^{2+}$ uptake was measured as in ref. 4. HmT toxin was UV irradiated for 60 min. The final toxin concentration used in the assay was 10 ng/ml. Values are the average of four experiments.

firmed that all of the toxin and analog preparations had biological activity by using the calcium uptake bioassay (as in the Table).

HmT toxin is highly specific for the mitochondria of cms T corn. The maternally-inherited characters of cytoplasmic male sterility and toxin sensitivity are tightly linked and almost certainly the gene(s) are located on the mitochondrial DNA. Recently a unique sequence of DNA has been isolated from cms T corn mitochondrial DNA [2] which codes for a polypeptide [3] uniquely translated in the mitochondria of cms T corn. This polypeptide could be involved in the expression of cytoplasmic male sterility and could also function as the toxin receptor accounting for the specificity of toxin action. Model membrane studies incorporating this polypeptide plus HmT toxin may yield information on how this toxin is involved in increasing membrane permeability.

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