INHIBITION OF THE K^+-STIMULATED ATPase OF MAIZE ROOT MICROSOMES BY HELMINTHOSPORIUM MAYDIS RACE T PATHOTOXIN*

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SUMMARY: The microsomes obtained by centrifugation between 12,000 g and 80,000 g of an homogenate of maize root tips contain K^+-stimulated ATPase activity. Pre-incubation of the microsomes from roots of cytoplasmic male sterile (cmsT) plants with partially purified pathotoxin from Helminthosporium maydis Race T strongly inhibits the K^+ stimulation; the same treatment of microsomes from normal roots has no effect. We suggest this inhibition is closely related to the mode of action of H. maydis Race T pathotoxin.

INTRODUCTION

The southern leaf blight of corn, which reached epiphytotic proportions in the United States in 1970, is caused by a fungus, Helminthosporium maydis Nisikado and Miyaka Race T, which selectively attacks maize plants bearing the cytoplasmic gene cmsT for male sterility (1). Several plant pathogens, including H. maydis race T, produce pathotoxins that damage plant cells, producing disease symptoms (2). In some cases the host-plant specificity of the pathotoxin is identical to that of the pathogen (3). Membrane permeability changes, resulting in ion leakage, are caused by the host-specific pathotoxins produced by H. victoriae (2), Periconia circinata (4) and H. maydis race T (5,6) and perhaps others. The nature of the interaction of the pathotoxins with plant cells has not been determined. Miller and Koeppe (7) observed that the H. maydis race T pathotoxin caused swelling and other changes in mitochondria from cmsT plants, although mitochondria from normal plants were unaffected, but this cannot

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account for the rapid change in membrane permeability caused by the pathotoxin. This communication is to report that the H. maydis Race T pathotoxin inhibits the K^+-dependent ATPase from roots of Tcms maize plants but has no effect on the enzyme from normal plants.

MATERIALS AND METHODS

Ethyl acetate-soluble pathotoxin was produced as described previously (1,6) and purified further by thin-layer chromatography on silica gel GF^{254} (E. Merck, Darmstadt, Germany) with a solvent composed of chloroform: methanol 9:1 v/v. The portion of the plates from Rf 0 to 0.4 was eluted with ethyl acetate, yielding a partially purified pathotoxin which was stored at -20° in ethyl acetate solution. Before use, the solvent was evaporated and the residue redissolved in water to a volume corresponding to the original culture volume from which that aliquot was derived. The pathotoxin assay used in purification was stimulation of ion leakage from roots of cmsT plants (6). Seed of the maize variety B37, with normal (N) or male-sterile (T) cytoplasm was obtained from Dr. Peter A. Peterson, Department of Agronomy, Iowa State University. The seeds were germinated and cell fractions from root tips were prepared essentially as described by Leonard and Hansen (8) except that Na_2-EDTA was used in the solution in which the roots were ground. Assay for ATPase activity was conducted as described by Leonard and Hansen (8) with minor modification.

RESULTS AND DISCUSSION

K^+-stimulated ATPase activity is found in the 80,000 pellet, (designated microsomes) from maize root tips. The corresponding fraction from oat roots has been shown to contain fragments of plasma membrane and the K^+-stimulated ATPase is strongly implicated in K^+ transport (9). Addition of pathotoxin to the microsomal preparation from maize roots strongly inhibited, and sometimes completely abolished, K^+-stimulated ATPase activity (Table I). That the inhibition is actually due to the pathotoxin and not to impurities in the preparation is indicated by the specificity, since ATPase from N plants is unaffected.

TABLE I

Effect of H. maydis Race T pathotoxin on K^+-stimulated ATPase activity of maize root microsomes.

Cytoplasm	Pathotoxin	K^+-stimulated ATPase activity Pi, μ moles/hr/g fresh tissue Experiment Number I	II	III	IV	V
N	-	2.60	2.41			2.41
N	+	2.60	2.30			2.28
T	-	3.08	2.00	2.43	2.41	2.50
T	+	0.00	0.73	0.27	0.39	0.00

Reaction mixtures contained 0.75 μ moles ATP (Tris salt, pH 6.8); 0.875 μ moles $MgCl_2$, when added; 12.5 μ moles KCl, when added; from 4.0 to 9.0 μ moles Tris-HCl buffer, pH 6.8 and enzyme, approximately 90 μg protein, in a final volume of 1.5 ml. Incubation was at 30C for 30 min.

In addition, toxin that has been inactivated (as shown by the root ion leakage assay) by being dissolved in $CHCl_3$:AcOH 98:2 v/v, then evaporated to dryness *in vacuo* and redissolved in water, has no effect on the ATPase activity.

These results suggest that the *H. maydis* race T pathotoxin combines with and inactivates a K^+ transport system in the plasma membrane of root cells from maize plants having the cmsT cytoplasmic gene for male sterility. As a result, the cells are unable to maintain the necessary intracellular K^+ concentration and extensive metabolic alterations ensue. This is consistent with the rapidity with which electrolyte leakage can be detected after exposure to toxin (6).

An interesting implication of this finding is that the cytoplasmic gene cmsT has a gene product located in the plasma membrane, either the K^+-stimulated ATPase or something closely associated with it.

Further work is in progress to determine the differences between the K^+-dependent ATPase from N and T plants. Use of the inhibition as an assay for toxin activity to facilitate purification of the toxin also is being investigated.

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