A NOVEL ELASTASE-LIKE PROTEIN FROM THE GYPSY MOTH IS INVOLVED IN THE PROTEOLYTIC ACTIVATION OF *BACILLUS THURINGIENSIS* TOXINS

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Abstract

Crystal proteins (Cry) from the bacterium *Bacillus thuringiensis* (Bt) are known for their insecticidal activity. The most widely used Bt-based bioinsecticides in agriculture and forestry in North America are based on the HD-1 isolate of *B. thuringiensis* subsp. *kurstaki*, which produces three distinct insecticidal proteins called Cry1Aa, Cry1Ab and Cry1Ac. After ingestion by susceptible insects, the crystals are solubilized in the alkaline environment of the midgut; protoxins with an apparent size of 130-140 kDa are released and proteolytically processed by midgut proteases, producing activated toxins with an apparent size of 55-60 kDa. The activated toxins bind to specific receptors on the midgut epithelium membrane. An additional proteolytic cleavage of the toxins is believed to be a prerequisite for insertion of the toxins into the membrane and for pore formation.

In this study, an elastase-like protease with an apparent size of 50 kDa that binds Cry1A toxins was found in gypsy moth larval digestive fluids. The protein was purified by a calcium precipitation step followed by ion-exchange and gel filtration chromatography. In contrast to gypsy moth trypsin and chymotrypsin, the elastase-like protease interacts with the calcium-mimic dye Stains-all and is recognized by antibodies raised to the putative Bt toxin receptor (BTR-270) purified from gypsy moth brush border membrane vesicles. The digestion of Cry1A protoxin using purified gypsy moth trypsin, chymotrypsin, and elastase generates products that are different in size, suggesting that the activities of these products may be different. In addition, the elastase-like protease binds trypsin-activated Cry1A toxin and produces an activated toxin smaller than trypsin or chymotrypsin, implying that it may be involved in the final step of activation of the Bt toxin, which triggers formation of a membrane-competent pre-pore structure necessary for insertion of the Bt toxin into the membrane and for toxicity.