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## Mode of Action of a Specific Insecticidal Protein from *Bacillus thuringiensis*

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*Bacillus thuringiensis* (Bt) is a gram-positive soil bacterium that produces, during sporulation, crystalline inclusions consisting of highly specific insecticidal proteins called insecticidal crystal protein (ICP),  $\delta$ -endotoxin or Cry protein, which are toxic to the larvae of lepidopteran, dipteran, and coleopteran insects. An outline of the action of ICPS is as follows. The ICPS are ingested by susceptible insect larvae and dissolved in the midgut under generally alkaline and reducing conditions. The solubilized ICPS are processed by gut processes into the active forms, which bind to the surface of midgut epithelial cells and form a leakage channel in the cell membrane. Colloid-osmotic swelling and lysis of the cell result in death of the larvae. Thus, *B. thuringiensis* has been used, as bioinsecticides, in agriculture, forest management and mosquito control.

*B. thuringiensis* produces different ICPS among subspecies. *B. thuringiensis* subsp. *israelensis* produces dipteran-specific ICPS, CryfA, Cry4B and Cry11A, and non-specifically cytotoxic Cyt1A.

A 130-kDa protoxin of Cry4A, a dipteran-specific ICP, was processed into 20- and 45-kDa fragments through a 60-kDa intermediate by the midgut

proteases of larvae of mosquito *Culex pipiens* both *in vitro* and *in vivo*. Neither the 20-kDa nor the 40-kDa fragment was toxic against *C. pipiens* larvae but the significant toxicity was observed when both the fragments coexisted. The two fragments are associated with each other. Thus, it is demonstrated that the complex consisting of the 20- and 45-kDa fragments is an active form of Cry4A.

Some ICPS including Cry4A bind to the surface of epithelial cells of the larval midguts of mosquito and silkworm. They also bind brush border membrane vesicle (BBMV) from the larval midguts. However, their insecticidal specificities are not necessarily interpretable based on their characteristics in the initial binding step mentioned above. Another binding state should be involved in determining the insecticidal specificities of the ICPs.

Production of ICP in *B. thuringiensis* cells is regulated primarily at the transcriptional level. The transcriptional regulation of the cry4A gene is somewhat more complicated than other cry genes. It is strongly suggested that the cry4A transcription is under the control of three types of  $\delta$  factors