# Domain I Plays an Important Role in the Crystallization of Cry3A in *Bacillus thuringiensis*

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## Abstract

The insecticidal bacterium *Bacillus thuringiensis* synthesizes endotoxin Cry proteins of two size classes, 135 and 70 kDa, and both form crystalline inclusions in cells after synthesis. Crystallization of 135-kDa proteins is due to intermolecular attraction of regions in the C-terminal half of the molecule, and the N-terminal half fails to crystallize when synthesized in vivo. Alternatively, endotoxins of the 70-kDa class such as Cry2A and Cry3A, which correspond to the N-terminal half of 135-kDa molecules, crystallize readily after synthesis. Cry molecules of this size class consist of three principal domains, but the domains responsible for crystallization are not known. To identify these domains, chimeric proteins were constructed in which Cry3A Domains I or III, or I and III were substituted for the corresponding domains in truncated Cry1C molecules. Cry1C molecules with only Cry3A Domain III did not crystallize, whereas when Cry3A Domains I and III, or Domain I alone, were substituted, large inclusions were obtained. Except for the chimera consisting of Cry3A Domains I and III and Cry1C Domain II, most chimeras were not as stable as wild-type Cry3A or truncated Cry1C. These results show that Cry3A Domain I plays an important role in its crystallization in vivo.


**Index Entries:** *Bacillis thuringiensis*; protein crystallization, insecticidal proteins domain structure; domain function; chimeric proteins.

## 1. Introduction

The various subspecies of *Bacillus thuringiensis* produce a wide variety of insecticidal proteins known as Cry or Cyt δ-endotoxins that crystallize in the host cells after synthesis ***(1)***. These proteins are the active ingredients in bacterial insecticides used to control many important agricultural pests and vectors of animal and human diseases ***(2)***. After ingestion by an insect, crystals composed of Cry proteins dissolve in the insect midgut and are cleaved by proteases, releasing a toxin core. This activated toxin binds to proteins on the apical portion of midgut microvilli, and then inserts creating pores that cause cell lysis and insect death ***(1–3)***.

Cry proteins can be divided into two classes based on mass, those of, respectively, 135 kDa and 70 kDa ***(3)***. The 135-kDa endotoxins such as Cry1 proteins active against lepidopterans are protoxins in which the N-terminal half contains the actual toxin, which is activated and released by proteolytic cleavage in the insect midgut. The C-terminal half of Cry1 molecules is highly conserved among Cry1 proteins, but has no toxic function. Its putative function is to facilitate crystallization of toxin molecules after synthesis ***(4,5)***. This half of the molecule contains most of the cysteine residues, and it has been suggested that these form intermolecular disulfide bridges that stabilize the crystal ***(6)***. When truncated Cry1 molecules consisting of the N-terminal half are synthesized in *B. thuringiensis* or other bacteria, they fail to crystallize ***(7–9)***.

In contrast to 135-kDa proteins, 70-kDa Cry proteins lack the C-terminal crystallizing domain, and thus correspond to the N-terminal half of Cry1 proteins. Despite the absence of a crystallizing

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