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PURIFICATION OF CRYTOXIN FROM *Bacillus thuringiensis*

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ABSTRACT

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of Crytoxin activated toxin purified by DEAE-52 column chromatography produced a sharp band on gel. The molecular weight of the purified crytoxin peptide was estimated to be about 65 kda.

Keywords: Crytoxin, Gel electrophoresis (SDS-PAGE) and *Bacillus thuringiensis.*

INTRUDOCTION

Bacillus thuringiensis, a soil dwelling bacterium synthesizes insecticidal crystal proteins (ICPs) which are also called delta-endotoxins or crytoxins. These are active against lepidopteran, dipteran and coleopteran larvae[1,2]. Different workers isolated and purified this toxic protein from the bacterium in order to study their mode of action and other mechanism[3-6]. Here the purification of cry delta endotoxin from local Bt strains using simple and conventional chromatographic techniques - gel filtration and ion-exchange chromatography.

Bt toxin production and extraction:

A strain of *Bacillus thuringiensis* known to produce toxin protein was grown in liquid medium at 30°C with continuous shaking at 200 rpm until complete sporulation. The spore crystals were collected by centrifugation at 700 rpm at 4°C for 5 minutes. The pellet was washed twice with deionized water, once with 0.5 M NaCl and once with 10 mM EDTA. The suspension was centrifuged and the pellet was stored at -20°C. The crystals pellet (spores) was resuspended in equal volume of alkali buffer (50 mM sodium carbonate, 10mM dithiothreitol, pH 10-12) and incubated overnight at 37°C. The sample was centrifuged on the following day and the suspension contained solubilized toxin protein.

Determination of protein concentration:

The concentration of protein was measured by Bio-Rad Protein Assay method using BSA as the standard, and the protein in column-elute fraction was also monitored spectrophotmetrically at 280 nm.

Purification of cry protoxin

Gel filtration on Sephacryl S-300 column

The crude toxin protein extract (18 ml containing 300 mg protein) was loaded on Sephacryl S-300 column (2.5 x 100 cm) previously equilibrated with 3 column volume of 1M urea solution. Fractions (3 ml) were collected in test tubes at a flow rate of 60 ml/hour by an automatic fraction collector. Absorbance of each fraction at 280 nm were measured spectrophotometrically and their purity were checked by SDS-PAGE. Through this purification step about 50 mg protein was obtained. These fractions contained impure protein as they gave more than one band on the polyacrylamide gel. For further purification after activation with trypsin, these fractions were subjected to DEAE-52 column chromatography.

Activation of protoxins

The Crytoxin containing fraction from Sephacryl S-300 column was pooled, dialysed against 5 L of 0.1M Na_2CO_3 buffer. Activation of protoxin for the generation of toxin peptide was performed by incubating it at 37°C with trypsin (stock solution made to a concentration of V/ml in deionised distilled water) in the mass ratio of 1:20.

DEAE-52 ion-exchange chromatography

The activated fraction was dialysed against 5 L of 0.1M phosphate buffer, 2% glycerol) for 18 hours at 4°C. After centrifugation, the supernatal (ml containing 19mg) was loaded on DEAE-52 column (5 ml gel bed) previously equilibrated with 60 ml of 0.1M phosphate buffer, 2% glycerol, pH 7.5. The separation of protein from DEAE-52 column was achieved by gradient elution with the buffer containing 1M KCl. Fractions (3 ml) were collected at a flow rate of 10 ml/hour by an automatic

fraction collector and absorbance of each fractions spectrophotometrically analysed at 280 nm. The SDS-PAGE results indicated that the pooled fraction contained pure protein, as they produced a sharp band on the gel. The molecular weight of the purified crytoxin peptide was determined by comparing its mobility with those of marker proteins of known molecular weights on SDS-PAGE and was estimated to be about 65 kda.

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