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Reconstruction of *Bacillus thuringiensis* ssp. *israelensis* Cry11A Endotoxin from Fragments Corresponding to Its N- and C-Moieties Restores Its Original Biological Activity

L. P. Revina[1], L. I. Kostina[1], L. A. Ganushkina[2], A. L. Mikhailova[1], I. A. Zalunin[1]*, and G. G. Chestukhina[1]

[1]Scientific Research Institute for Genetics and Selection of Industrial Microorganisms, I Dorozhny Proezd 1, Moscow 117545, Russia; fax: (7-095) 315-0501; E-mail: zalunin@genetika.ru
[2]Marcinovsky Institute of Medical Parasitology and Tropical Medicine, Sechenov Moscow Medical Academy, Bolshaya Pirogovskaya ul. 2, Bldg. 3, Moscow 119992, Russia

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Abstract—Subtilisin hydrolyzes Cry11A endotoxin (of 70 kD) produced by *Bacillus thuringiensis* ssp. *israelensis* to fragments of 33- and 36-kD, which correspond to N- and C-terminal halves of the endotoxin molecule. Thermitase (a serine protease from *Thermoactinomyces vulgaris*) and insect gut proteases from *Diptera* and *Lepidoptera* exhibit the same hydrolytic effect on Cry11A. Hydrolyzates maintain high toxicity with respect to larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex pipiens*. The 33- and 36-kD Cry11A endotoxin components purified by ion-exchange chromatography from the subtilisin hydrolyzate were inactive; however, equimolar mixture of these proteins exhibited almost the same activity as the initial hydrolyzate.

Key words: Cry11A endotoxin, *Bacillus thuringiensis* ssp. *israelensis*, limited proteolysis, mosquitocidal activity

δ-Endotoxins from *Bacillus thuringiensis* (Cry-proteins) constitute a large class of proteins forming crystal-like inclusions in sporulating cells of various strains of this microorganism; they are toxic for larvae of various insect species [1]. Usually δ-endotoxins are proteins of molecular mass 130-145 kD. Their bioactivation occurs in the insect gut by conversion into "true toxins" of molecular mass of ~70 kD involving proteolytic processing [2]. Some entomocidal proteins have similar molecular mass of 65-70 kD and they share structural similarity with true toxins [1]. In spite of significant differences in primary structure and specificity of the entomocidal effect, δ-endotoxins share similarity in secondary and tertiary structures. The true toxins and 65-70 kD endotoxins consist of three domains: N-terminal domain, which contains α-helices, and two domains enriched with elements of β-structure [3, 4]. These domains are functionally autonomic, and each of them plays its own role in the overall toxic effect. The β-structural domain interacts with receptors located in membranes of the target cells, whereas the α-structural domain is involved in formation of transmembrane pores or ion channels [1].

In most cases, true toxins are resistant to subsequent proteolysis. However, some of them (Cry4B, Cry9A) [5] and also most of 65-70 kD endotoxins (Cry2A, Cry3A, Cry11A) [6-8] undergo deeper proteolysis, and the degree of this proteolysis varies in various toxins. The fact that these proteins can lose various and sometimes significant parts of their molecules during proteolytic digestion by gut proteases but do not lose their biological activity suggests the existence of certain differences in their action mechanisms. In this study, we have investigated in detail the process of limited proteolysis of Cry11A endotoxin and the biological activity of the resultant fragments.

MATERIALS AND METHODS

Preparation of Cry11A endotoxin. *B. thuringiensis* ssp. *israelensis* strain B-2395 was grown using a liquid medium containing 0.5% tripcasin (Human, Hungary), 0.5% glucose, and 0.2% yeast extract (Serva, Germany) [9]. Endotoxin crystals were prepared from sporulated culture using a biphasic xylene–water system [9]. Cry11A endotoxin was isolated by the method of selective extraction [10]. Initially crystals of B-2395 strain

* To whom correspondence should be addressed.

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were suspended (5 mg/ml) in 50 mM sodium carbonate buffer, pH 9.8, containing 10 mM DTT (Serva) and 1 mM EDTA and incubated at 20°C for 1 h. The supernatant obtained by centrifugation at 5500*g* in a K 23 centrifuge (JANETZKI, Germany) for 30 min contained Cry4B and CytA endotoxins and products of their proteolysis. The sediment containing Cry11A endotoxin was treated at 20°C for 30 min with a mixture of protease inhibitors (1 mM diisopropyl fluorophosphate and 10 mM EDTA) and dissolved in 50 mM NaOH, pH 12, containing 5 mM EDTA. The pH was then reduced to 8.0 with 1 M Tris-HCl. The supernatant obtained after centrifugation of this suspension at 5500*g* in a K 23 centrifuge for 20 min contained Cry11A and products of its proteolysis.

Protease extraction from insect larvae guts. Midguts were dissected from 3rd instar larvae of *Aedes togoi*, *Anopheles atroparvus*, *Culex pipiens* (*Diptera*), and *Lymantria dispar* (*Lepidoptera*). They were then incubated in 20 mM sodium phosphate buffer, pH 7.5, containing 50 mM NaCl for 2 h on ice with stirring. The mixture was then centrifuged at 8000*g* in a Eppendorf centrifuge (Eppendorf AG, Germany) for 5 min. The resultant supernatants containing a wide spectrum of compounds secreted by larval gut epithelium were used as a source of proteolytic enzymes of these insects and in this paper defined as the preparation of gut proteases. To characterize proteolytic activity of these preparations we measured their tryptic activity with *p*-nitroanilide benzoyl-D,L-arginine as substrate [11]. The amount of activity units per 1 ml of extract was calculated using the formula:

$$A_{410} \cdot V_{\text{reaction mixture}}/8.2 \cdot V_{\text{sample}} \cdot t,$$

where A_{410} is optical density at 410 nm, V is volume (ml), t is reaction time (min), and 8.2 is absorbance of 1 mM *p*-nitroaniline at light path of 1 cm.

Limited proteolysis of Cry11A endotoxin. Solution of endotoxin Cry11A in 50 mM Tris-HCl buffer, pH 8.5, was treated at 20°C with thermitase (a serine protease from *Thermoactinomyces vulgaris* kindly presented by A. L. Osterman), subtilisin (Serva), trypsin (Sigma, USA), and preparations of larval gut proteases from *Aedes togoi*, *Anopheles atroparvus*, *Culex pipiens* (*Diptera*), and *Lymantria dispar* (*Lepidoptera*). The ratios of enzyme/toxin were 1 : 100 or 1 : 500 and the incubation time was 30 min. Gut protease preparations (0.005 or 0.015 U of tryptic activity per 1 mg of toxin) were incubated with the toxin for 3 h. Hydrolysis was stopped by adding 1 mM diisopropyl fluorophosphate.

Separation of products of limited proteolysis of Cry11A endotoxin. Subtilisin hydrolyzate of Cry11A endotoxin was applied onto Mono Q ion exchanger (0.5 × 5 cm, in FPLC mode, Pharmacia, Sweden) equilibrated with 50 mM Tris-HCl, pH 8.8, containing 1 mM DTT. The hydrolyzate components were eluted by a linear gradient of 0-0.8 M NaCl in the same buffer; at the moment of peak elution, the gradient was retarded.

Electrophoresis. PAGE in the presence of SDS was carried out by the method of Laemmli [12] using 10% gels and the following protein molecular weight markers: BSA (66 kD), ovalbumin (45 kD), glyceraldehyde-3-phosphate dehydrogenase (36 kD), carboanhydrase (29 kD), soybean trypsin inhibitor (20 kD), and cytochrome *c* (12 kD).

Determination of N-terminal amino acid sequence. Products of limited proteolysis of Cry11A endotoxin were separated by PAGE in the presence of SDS and transferred onto Millipore Immobilon-P^{SQ} membrane (USA). The N-terminal amino acid sequence for corresponding bands was determined an Applied Biosystems 470A model gas phase sequencer (USA).

Assay of biological mosquito-larvicidal activity. Mosquito larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex pipiens* were used as insect test species. Cry11A endotoxin and products of its limited proteolysis were sedimented by adding 1 M citric acid to pH 4.5 and incubated in a refrigerator for 7 days. From these suspensions, six dilutions (with fourfold concentration interval) were prepared using 10 mM sodium citrate, pH 4.5. Each dilution was studied in triplicate. All experiments were carried out at 26°C. In each experiment, 25 mosquito larvae of the 2nd instar were placed in glass vials containing 50 ml of a certain dilution. (Control 25 larvae were placed into vials containing 50 ml of 10 mM sodium citrate, pH 4.5.) The number of surviving larvae was calculated after treatment for 24 h.

Mosquito-larvicidal toxicity was expressed as LC_{50} value, representing the concentration of a toxin preparation causing death of 50% of the larvae.

RESULTS

Proteolytic cleavage of Cry11A endotoxin. Incubation of Cry11A endotoxin with thermitase at 20°C for 1 h (at the ratio enzyme/substrate 1 : 500) resulted in formation of two fragments with molecular masses of 33 and 36 kD (Fig. 1d). Determination of the N-terminal sequence of these electrophoretically separated proteolytic products revealed that the 33 kD fragment begins from the sequence APAQIA. The latter differs by one residue (Q32) from the sequence APALIA corresponding to the region between residues 29 and 34 of the primary structure of Cry11Aa isolated from another strain of ssp. *israelensis* [13]. Thus, the 33 kD fragment represents the N-terminal half of the Cry11A molecule (the fragment begins from A29), and thermitase hydrolyzes the I28–A29 bond. Cleavage of small N-terminal fragments occurs during processing of 130 kD endotoxins [3]. It is possible that these initial sites of Cry protein molecules are not structured and are easily susceptible to proteolytic enzymes.

A 36-kD fragment formed from Cry11A endotoxin has the following N-terminal sequence DIINQILTAP. Consequently, it originates from hydrolysis of the peptide bond R360–D361 and corresponds to the C-terminal half of the endotoxin molecule [13]. This means that thermitase cleaves Cry11A endotoxin roughly into two halves.

At the ratio thermitase/substrate 1 : 100 (w/w) a 25-kD fragment appears in the hydrolyzate (Fig. 1e). Its N-terminal sequence EKVRTEV suggests that appearance of this fragment can be attributed to cleavage of peptide bond M71–E72 in the 33-kD fragment. The scheme in Fig. 2 summarizes the effect of thermitase on Cry11A endotoxin.

Subtilisin exhibits the same pattern of hydrolysis (Fig. 4). Fragments of molecular mass of 36, 33, and 25 kD were obtained during the action of this enzyme at 20°C for 30 min at the enzyme/substrate ratio 1 : 100 (w/w). The N-terminal sequences for the 33-kD (LIAVAPIA) and 36-kD (TFYQNPN) fragments imply that in this case hydrolysis involves the A31–L32 and Q346–T347 bonds.

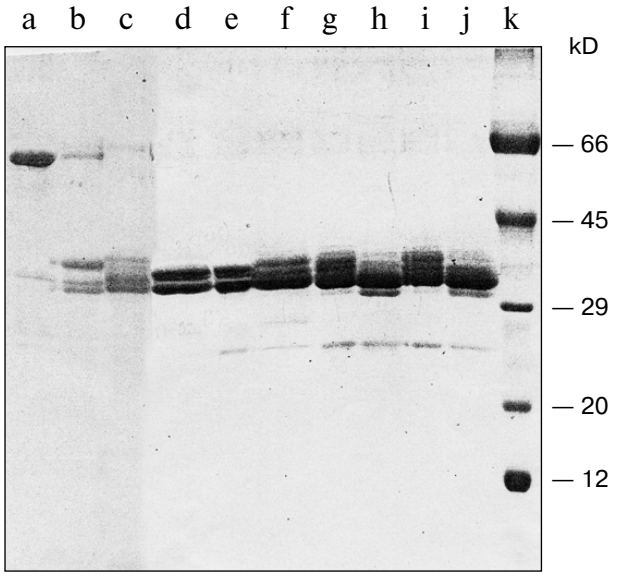

Fig. 1. Limited proteolysis of Cry11A endotoxin by trypsin, thermitase, and insect gut proteases. Cry11A endotoxin (a) in 50 mM Tris-HCl buffer, pH 8.5, was treated: by trypsin at the ratio 1 : 100 (w/w) (b); by thermitase at ratios 1 : 500 (d) and 1 : 100 (e); by gut proteases from larvae of *Lymantria dispar* (0.005 U/mg protein) (c), of *Aedes togoi* (0.005 U/mg protein) (f), of *Anopheles atroparvus* (0.005 U/mg protein (g) and 0.01 U/mg protein (h)), and of *Culex pipiens* (0.005 U/mg protein (i) and 0.015 U/mg protein (j)). Aliquots of hydrolyzates (20 μl) were precipitated with trichloroacetic acid (final concentration 7%), redissolved in sample buffer, and subjected to PAGE in 10% gel in the presence of SDS. The following molecular weight protein standards (k) were used: BSA (66 kD), ovalbumin (45 kD), carboanhydrase (29 kD), soybean trypsin inhibitor (20 kD), and cytochrome *c* (12 kD).

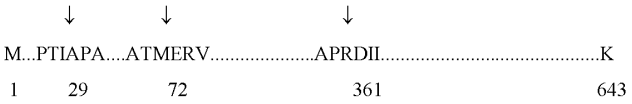

Fig. 2. Scheme of limited proteolysis of Cry11A endotoxin by thermitase.

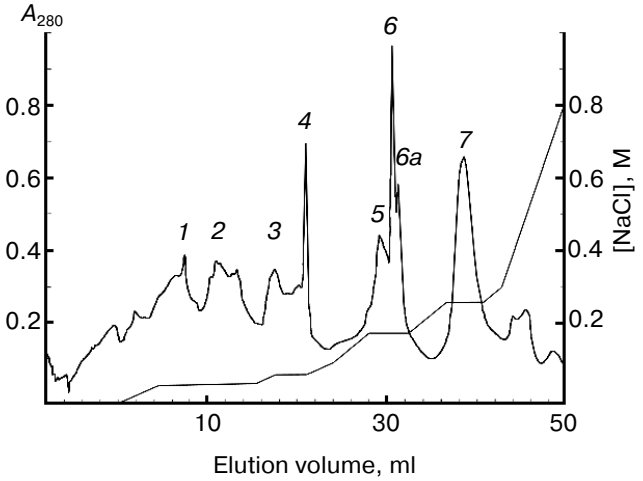

Fig. 3. Ion-exchange chromatography of subtilisin hydrolyzate of Cry11A endotoxin. The subtilisin hydrolyzate of Cry11A endotoxin was applied onto a Mono Q column equilibrated with 50 mM Tris-HCl buffer, pH 8.8, containing 1 mM DTT. Proteins were eluted by a linear gradient of NaCl (0-0.8 M) in the same buffer; at the moment of peak elution, the gradient was retarded. Numbers 1-7 indicate the main chromatographic peaks.

Preparations of mosquito larvae gut proteases hydrolyze Cry11A endotoxin; this results in formation of 32-38-kD fragments (Fig. 1, f-j). Increase in gut enzyme concentration and incubation time increased the content of 32- and 33-kD components, which gradually began to dominate (Fig. 1, h and j). A minor amount of 25-kD component was also detected in the hydrolyzates.

Treatment of Cry11A endotoxin by proteases from larvae of *Lymantria dispar* resulted in appearance mainly of components with molecular masses of 33, 34.5, and 38 kD (Fig. 1c).

Evidently, insect proteases cleave the endotoxin molecule into two roughly equal N- and C-terminal halves.

Chromatography of proteolytic products of Cry11A endotoxin treated with subtilisin. A subtilisin hydrolyzate of Cry11A endotoxin was fractionated on a Mono Q column at pH 8.8 in the presence of 1 mM DTT. Figures 3 and 4 show results of chromatography and electrophoretic study of collected fractions. Major peaks obtained after protein elution contained the 36 kD fragment (peaks 3 and 4), the 25 kD fragment (peaks 6 and 6a), the 33 kD fragment (peak 7), and a mixture of 33- and 36-kD fragments (peaks 2 and 5). According to data of electrophore-

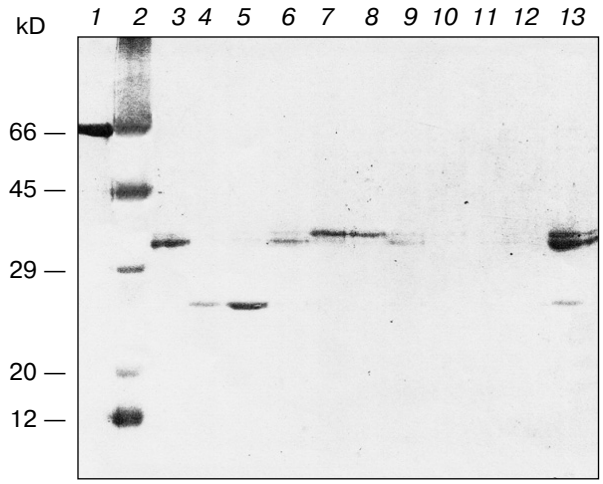

Fig. 4. Electrophoregram of fractions obtained during ion-exchange chromatography of subtilisin hydrolyzate of Cry11A endotoxin. Aliquots (20 μl) of fractions obtained during chromatography of Cry11A subtilisin hydrolyzate were loaded into the following lanes of a 10% polyacrylamide gel: *3*) peak 7; *4*) peak 6a; *5*) peak 6; *6-10*) peaks 5-1; *11*, *12*) non-adsorbed proteins; *1*) Cry11A endotoxin; *13*) initial hydrolyzate; *2*) protein standards.

sis under denaturing conditions, the resultant preparations contained small contaminations of other components.

Mosquito-larvicidal activity of Cry11A and its proteolytic products with respect to larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex pipiens*. In term of LC_{50} value the toxicity of initial preparation of Cry11A endotoxin for *Aedes aegypti* larvae varied from 10.8 to 17.9 ng/ml (table). A similar LC_{50} value (8.6 ng/ml) was obtained for *Culex pipiens* larvae. This suggests that Cry11A endotoxin exhibits comparable activity against these insect species, whereas *Anopheles stephensi* larvae were more resistant to this toxin (LC_{50} of 135 ng/ml) (table).

We also studied toxicity of total hydrolyzates obtained by treating Cry11A endotoxin with trypsin, subtilisin, and preparations of gut proteinases from *Aedes togoi*, *Anopheles atroparvus*, and *Culex pipiens*. Data of the table show that limited proteolysis insignificantly influenced toxicity (and sometimes even potentiated it).

Study of biological activity of the isolated fragments of Cry11A endotoxin revealed (table) that the 33-kD fragment corresponding to the N-terminal half of the endotoxin molecule was 17 times less toxic for *Aedes aegypti* than the intact protein. The C-terminal half of 36 kD was even less toxic than the N-terminal peptide—at concentration of 400 ng/ml it caused death of only 36% of larvae.

Mixing of both fragments at the ratio 1 : 1 completely restored the initial toxicity, the LC_{50} value for this mixture being 21.9 ng/ml.

DISCUSSION

Limited proteolysis of Cry11A endotoxin. Endotoxins produced by *Bacillus thuringiensis* act in the gut of insect larvae and, consequently, they are subjected to severe treatment by proteolytic enzymes. In most cases high molecular weight Cry proteins (130-145 kD) undergo processing by gut proteases; this results in formation of the true toxins (of lower molecular weight of 65-70 kD), which are rather resistant to subsequent proteolysis [2].

However, in some cases (e.g., Cry4B and Cry9A [5]) the true toxins readily undergo subsequent proteolytic conversions. δ-Endotoxins of molecular mass of 65-70 kD, which share structural similarity with the true toxins (e.g., Cry2A, Cry3A, and Cry11A [6-8]), may be also subjected to proteolysis. Almost all of these endotoxins are subjected to additional proteolysis in the N-terminal α-helical domain. However, in Cry11A endotoxin the hydrolyzable bond is located in the second domain. In this case, proteolysis results in two basically equal fragments (about 35 kD) corresponding to the N- and C-halves of the molecule.

Results of the present study (Fig. 1) and literature data [8, 14-16] indicate that mode of Cry11A hydrolysis does not strictly depend on specificity of the proteolytic enzymes. Differences in the action of these enzymes mainly affect subsequent "nibbling" of the resultant halves of the initial molecule. Insect proteolytic enzyme complex has "greater proteolytic facilities" than individual enzymes because it contains several proteases, exhibiting trypsin-, chymotrypsin-, and elastase-like and also exopeptidase activities [15]. High pH values in the gut lumen (pH ≥ 10) may also cause partial denaturation of a protein molecule and this facilitates the process of proteolytic cleavage. However, even use of extracts from the gut of various insects does not alter the general mode of Cry11A proteolysis: proteolysis of the endotoxin molecule yields two almost equal parts (halves) (Fig. 1, see also [15, 16]). The specific sets of proteolytic enzymes secreted into the gut of various insect species show small differences in number and molecular mass of the fragments formed. This suggests that the proteolytic site is determined by the structure of the entomocidal protein.

Molecules of the true toxins of high molecular weight Cry-proteins and Cry-proteins of 65-70 kD consist of three tightly bound domains so that limited proteolysis (if it is possible) occurs at some loops connecting elements of these domains [3, 5, 17]. In most cases intradomain loops are inaccessible to proteolytic enzymes [3]. So, intradomain proteolysis requires special conditions. For example, loosening of the N-terminal part of the sixth α-helix due to the presence of Gly (Cry4B endotoxin) or Pro (Cry9A endotoxin) results in increased sensitivity to proteolysis of the α5–α6 loop of these proteins [5]. Unfortunately, the three-dimensional structure of Cry11A endotoxin remains unknown. However, based on

Entomocidal activity (LC50, ng/ml) of Cry11A and products of its proteolysis

Preparation	LC_{50} (ng/ml) values obtained during 24-h observation for larvae of:		
	Aedes aegypti	*Anopheles stephensi*	*Culex pipiens*
Experiment 1			
Cry11A endotoxin	10.8	135.0	8.6
Products of Cry11A proteolysis by:			
subtilisin	7.0	41.8	6.9
gut proteases of:			
Aedes togoi	35.5	121.7	14.5
Anopheles atroparvus	n. d.	31.3	n. d.
Culex pipiens	n. d.	n. d.	5.7
Experiment 2			
Cry11A endotoxin	15.7	n. d.	n. d.
Products of Cry11A proteolysis by trypsin	5.4	n. d.	n. d.
Experiment 3			
Cry11A endotoxin	17.9	n. d.	n. d.
36-kD fragment	>400.0*	n. d.	n. d.
33-kD fragment	300.0	n. d.	n. d.
Equimolar mixture of 33- and 36-kD fragments	21.9	n. d.	n. d.

* 400.0 ng/ml is the maximal concentration studied; n. d., not determined.

theoretical model of closely related endotoxin Cry11Bb [18] we suggest that bonds hydrolyzed by trypsin (R360–D361 [8]), subtilisin (Q346–T347), thermitase (R360–D361), and insect gut proteases (T347–F348 and F348–Y349 [15, 16]) are located in the loop connecting β4 and β5 in the second domain of the Cry11A molecule.

The true toxins derived from proteolytic processing of Cry1A proteins may also be subjected to limited proteolysis, which involves the region of the second domain. The hydrolyzing bonds are located at the β5–β6 loop [19, 20]. However, in this case proteolysis occurs very slowly and with low yield. It also requires high protease concentrations and significant endotoxin denaturation. It is possible that unique proteolytic cleavage of Cry11A endotoxin is determined by accessibility of β4–β5 for proteolytic attack. In fact, the loop β4–β5 of Cry11A is longer than that of Cry1A (Fig. 5) and this may be a plausible reason underlying its accessibility to proteolysis [13, 21].

Gutierrez et al. [18] suggest that charged residues K353, D355, and H356 located in the β4–β5 loop of Cry11Bb endotoxin play an important role in its interaction with specific receptor. Comparison of amino acid sequences of Cry11 proteins demonstrates lack of the above mentioned residues in the Cry11Aa sequence (Fig. 5). It is possible that R360 and D361 located at the C-terminus of the β4–β5 loop play their role(s). Anyway, susceptibility of this region for proteolytic enzymes suggests its location on the surface of the protein molecule.

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          344                  364
Cry11Bb   VEQEITYNNKGDHSNSIVPAN

          343                  361
Cry11Aa   VRTQTFYQNP--NNEPIAPRD

          336                  347
Cry1Aa    PLFGN---------AGNAAPP
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Fig. 5. Multiple alignments of amino acid sequences of endotoxins Cry11Bb, Cry11Aa, and Cry1Aa within the region of the β4–β5 loop.

Sensitivity to proteolysis by subtilisin and gut proteases suggests that the region including residues 346-349 is also located on the surface of the protein molecule.

It should be noted that molecular masses of products of Cry11A endotoxin proteolysis by thermitase determined by electrophoresis under denaturing conditions differ from the values deduced on the basis of experimentally determined cleavable bonds. Unfortunately, we did not determine the C-terminal amino acid residues of the analyzed protein fragments. It is possible that other peptide bonds preceding R360–D361 bond in the Cry11A molecule are also sensitive to hydrolysis by thermitase. This might explain why molecular mass of the 33-kD fragment was lower than the expected value. In turn, the molecular mass of the 36-kD fragment was higher than the theoretically expected value (32 kD). It is possible that this discrepancy may be attributed to the presence of carbohydrates. Good evidence exists that toxins (including Cry11A) produced by *Bacillus thuringiensis* ssp. *israelensis* may be glycoproteins. This has already been documented in the literature [22, 23].

Biological activity of products of limited proteolysis of Cry11A endotoxin. High sensitivity of Cry11A endotoxin to *in vitro* proteolysis by certain individual proteases and protease complex secreted by mosquito larval gut epithelium suggests that in the insect gut this protein is readily converted into a set of peptides of 25-36 kD. Nevertheless, the mosquito-larvicidal activity of Cry11A endotoxin is rather high (table). This can be explained by two possible reasons: a) proteolytic products of Cry11A formed *in vivo* retain toxicity of the initial (intact) protein; b) the toxic effect may be attributed to a small proportion of endotoxin molecules which remain intact in the gut. Study of biological activity of Cry11A endotoxin hydrolyzates formed *in vitro* supports the first possibility [14-16]. In our study, Cry11A hydrolyzates lacking the intact protein retained larvicidal activity against mosquitoes of genera *Aedes*, *Anopheles*, and *Culex* (table).

There are controversial viewpoints on the requirement of proteolytic activation of 65-70-kD endotoxins [14-16]. Our data suggest that such activation clearly occurs. All hydrolyzates (except those obtained after the incubation with *Aedes togoi* gut extracts) were more toxic than the initial Cry11A endotoxin. Although the increase in toxicity was not very high (1.2-4.3-fold), it should be noted that in *in vivo* experiments we cannot determine actual biological activity of the full-sized protein because it is subjected to the effect of gut proteases during the course of the experiment. This may result in underestimation of the difference in biological activities of Cry11A endotoxin and its hydrolyzates provided that this difference actually exists.

Data of the table indicate that *Anopheles stephensi* larvae were 10-15 times less sensitive to Cry11A endotoxin than *Aedes* and *Culex* larvae. At the same time, the most significant toxin activation (by 4.3-fold) during Cry11A endotoxin treatment with gut proteases is shown in the case of *Anopheles stephensi* larvae. These results suggest that protease activity in the gut of these larvae is significantly lower than in other mosquito species; this reason may well explain lower sensitivity of *Anopheles stephensi* to Cry11A endotoxin.

Hydrolyzates of Cry11A endotoxin obtained after its treatment with *Aedes togoi* gut proteases exhibited less mosquitocidal activity (by 1.7-3.3 times) than the initial toxin. It is possible that toxin inactivation can be explained by additional cleavage of small fragment(s) from the main proteolytic products.

The existence of toxicity in hydrolyzates can be explained in two ways: 1) only one half of Cry11A is responsible for biological activity of Cry11A endotoxin; 2) limited proteolysis is accompanied by cleavage of peptide bonds, but this does not involve integrity of the molecule due to tight binding of proteolytic products by nonpeptide bonds.

In the present study, we have isolated products of limited proteolysis of Cry11A endotoxin and demonstrated that the 36-kD fragment basically lacks biological activity and the 33-kD fragment is 17-times less active than the intact toxin. These results clearly indicate that toxicity of Cry11A cannot be attributed to either half of the toxin molecule. This is consistent with modern concepts on the insecticide effect of *B. thuringiensis* endotoxins, which involves all domains of the toxin molecule into the total toxic effect [1]. Since the C-terminal half of the toxin molecule (36-kD fragment) lacks α-helical domain required for membrane perforation [1], it cannot cause insect death. However, the N-terminal half of the toxin molecule (33-kD fragment) contains an α-helical domain and a small part of the second domain (the size of this part we could not detect because we did not analyze C-terminal sequence of this fragment). It is possible that this part of the second domain might be responsible for low affinity receptor binding. Such binding would explain weak residual activity of the 33-kD fragment. However, it seems more probable that our preparations of 33- and 36-kD fragments are slightly contaminated by each other.

Mixing of 33- and 36-kD fragments at the equimolar ratio completely restored initial toxicity. Thus, dissociation of products of Cry11A endotoxin limited proteolysis may be reversed and the separated components exhibit high affinity to each other.

It is now accepted that interaction of Cry-proteins with plasma membrane of cell targets is accompanied by significant conformational changes [17, 24]. This results in a loss of tight contact between α-helices of the N-terminal domain and other domains of the endotoxin molecule. Some of these helices (presumably, the fifth and the sixth) are responsible for membrane perforation and subsequent formation of membrane pore or ion channel. Perhaps, in the case of Cry11A such rearrangement is

rather difficult and proteolytic cleavage of the peptide bond in the β4–β5 loop attenuating rigidity of the molecular structure facilitates this process. Since 33- and 36-kD fragments exhibit high affinity to each other, integrity of the toxin molecule is not affected by such proteolytic processing.

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