Note

Detection of ALMB-toxin in the Larval Body of Myrmeleon bore by Anti-N-terminus Peptide Antibodies

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Antibodies were raised against a synthetic antigen carrying the N-terminus peptide of ALMB-toxin, which had been isolated from the antlion, Myrmeleon bore, that exhibited high specificity to the toxin. Analyses with the antibodies showed the toxin to be present mainly at the larval stage and localized in a region from the thorax to abdomen of the larval body.

Key words: ALMB-toxin; antlion; Myrmeleon bore; antipeptide antibody; immunoblot

Larvae of some classes of brown lacewings, called antlions, create pitfalls to trap their prey. The observation that insects bitten by antlion mandibles became quiescent within a few minutes led us to assume that antlions inject a toxin into their prey. Anion-exchange in combination with hydrophobic column chromatography enabled us to purify a toxic substance from the crude venom of 700 larvae of Myrmeleon bore by monitoring the paralytic effect on male German cockroaches. The purified substance, named ALMB-toxin, is a single polypeptide with a molecular weight of about 170,000, exhibiting paralytic activity against cockroaches about 3,670-fold and 130-fold higher than Joro Spider Toxin-3 and Tetrodotoxin, respectively, on a molar basis.

ALMB-toxin may be used for crop protection by integrating the toxin-coding gene into insect-infecting viruses and plants as in the case of BT-toxins from Bacillus thuringensis. In extending our study to such goals, we considered that antibodies would be an essential tool for specific detection of the toxin and related peptides. However, the amount of toxin that can be isolated from a mass of antlions is so small that the toxin itself could not be used as the antigen for raising the antibodies. We therefore prepared a synthetic antigen carrying the N-terminus peptide of ALMB-toxin and raised antibodies in mice. We report here that the anti-N-terminus-peptide antibodies selectively reacted with the toxin and can be used for detecting toxin in the larvae of M. bore.

Five hundred milligrams of keyhole limpet hemocyanin (KLH, Calbiochem. Co., California, U.S.A.) were suspended in 20 ml of 0.02 m phosphate-buffered saline (PBS, pH 7.5), to which 150 mg of N-6-maleimido-caproyloxy succinimide (EMCS) dissolved in 3 ml DMF were then added at 0°C. The mixture was gently stirred overnight at 20°C. The reaction mixture was diluted by 10 ml of PBS and washed with ether. The aqueous phase, 1 N HCl was added to acidify the suspension while cooling on ice, and the precipitate was collected by centrifugation. The precipitate was suspended in 0.05% aqueous acetic acid, dialyzed against distilled water and lyophilized to yield 400 mg of EMCL-KLH as powder.

The N-terminus amino acid sequence of the purified ALMB-toxin was determined on a polyvinylidene fluoride (PVDF) membrane by an ABI 473A protein sequencer (Applied Biosystems, Chiba, Japan) as NH₂-SYENDAQIQIRNLK. According to this finding, an N-terminus peptide tailed by cysteine at the C-terminus (NH₂-SYENDAQIQIRNLKC-COOH) was synthesized by an ABI 430A peptide synthesizer (Applied Biosystems, Chiba, Japan). The integrity of the synthetic peptide was determined by an amino acid composition analysis with a JLC-300 analyzer (JEOL, Tokyo, Japan) and MALDI-TOFMS with a Voyager spectrometer (PerSeptive Biosystems, Tokyo, Japan). The peptide (14.5 mg) was conjugated with EMCL-KLH in 1.5 ml of a 0.02 m phosphate buffer (pH 7.5) containing 8 m urea and 0.9% NaCl at room temperature for 15 h. The reaction mixture was dialyzed against 8 m urea for 3 h, then against water and finally lyophilized, affording 28.7 mg of powder.

Male Balb/c mice (4 weeks old) were injected with 100 μl of a suspension of the conjugate in PBS (1.9 mg/ml) emulsified in an equal amount of complete Freund’s adjuvant. Two weeks later, the animals received a booster with the same amount of antigen emulsified in incomplete Freund’s adjuvant.

The crude toxin was collected and diluted by a 25 mM phosphate buffer (pH 7.5) as described. Whole or partial body protein samples were prepared by homogenizing the materials in PBS containing 20% trichloroacetic...
acid at 4°C, before collecting the precipitate by centrifugation. These samples were treated with the sample loading buffer (0.07 M Tris-HCl at pH 6.8 containing 5% 2-mercaptoethanol, 2% SDS, 5% sucrose and 0.004% bromophenol blue) for SDS polyacrylamide electrophoresis (PAGE). Protein concentration was determined by the method of Bradford.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out as described by Laemmli, the gels being stained with Coomassie Brilliant Blue R-250. Western blotting was carried out after the SDS-PAGE analysis. The peptides in the gel were electrophoretically transferred on to a PVDF membrane which was blocked by PBS containing 2% sodium azide and 2% bovine serum albumin. After washing the membrane with T-PBS (PBS containing 0.05% Tween-20) for 1 h, it was incubated for 1 h with the antiserum diluted 2,000-fold. After washing the membrane with T-PBS, the membrane was treated with the anti-mice IgG antibody conjugated with horseradish peroxidase (Boehringer Mannheim, Tokyo, Japan) and stained with PBS containing 0.06% 4-methoxy-1-naphthol, 20% methanol and 0.018% H$_2$O$_2$.

The crude venom and whole-body protein samples were separated by SDS-PAGE (Fig. 1A). A clear protein band was observed at the position of 170 kDa in the crude venom sample, while such a protein band was not clearly apparent in the whole-body protein sample. Immunoblotting allowed the detection of a single protein band in both the crude venom sample and in the whole-body sample (Fig. 1B). The detected protein was found to have the same N-terminus sequence as that of the

![Fig. 1. SDS-PAGE (A) and Immunoblot (B) Analyses of the Crude Venom and Whole-body Homogenate.](image1)

Lane 1, crude venom; lane 2, whole-body homogenate; lane 3, molecular weight markers. The marker proteins used were myosin (212 kDa), α-2-macroglobulin (170 kDa), β-galactosidase (116 kDa), transferrin (76 kDa), and glutamic dehydrogenase (53 kDa).

![Fig. 2. Immunoblot Analysis of the Stage-dependent Expression of ALMB-toxin.](image2)

Lane 1, larva; lane 2, pupa; lane 3, adult. Each lane was loaded with 30 μg of protein.

![Fig. 3. Immunoblot Analysis of the Protein Samples Prepared from Three Parts of Larvae of M. bore.](image3)

Lane 1, abdomen; lane 2, thorax; lane 3, head. Each lane was loaded with 30 μg of protein.
purified toxin, suggesting high selectivity of the antibodies.

To characterize the stage at which the toxin was produced, the larva, pupa and adult materials were analyzed by immunoblotting. The toxin could be clearly detected in the larval sample, but was only faintly and hardly observed in the pupal and adult preparations, respectively (Fig. 2), suggesting preferential secretion of the toxin at the larval stage. To preliminarily determine the distribution of the toxin in the insect body, the larva was roughly divided into the head, thorax and abdomen, and protein samples prepared from these parts were analyzed by immunoblotting. Figure 3 indicates that the toxin was distributed in the region ranging from the thorax to abdomen. We believe that this finding will become an important basis for identifying the toxin-secreting organ of *M. bore*.

In conclusion, we confirmed that ALMB-toxin was preferentially produced in the larval stage of *M. bore* by using anti-N-terminus peptide antibodies. We therefore consider the larval body to be the most suitable source for isolating mRNA containing the toxin gene message.

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References


