Larvicidal Activity of Mistletoe Lectin on Lepidopteran Pests: Mechanisms of Action

Mariam Gaidamashvili, and Eka Khurtsidze

Abstract*—Viscum album* berry lectin (MChbL) was tested against rustic shoulder-knot (*Apamea sordens*) and turnip moth (*Agrotis segetum*) larvae. MChbL affected larval development and survival at different growth stages. MChbL produced ca. 40% mortality of larvae when incorporated into an artificial diet at a level of 0.01% (w/w). MChbL affected larval gut proteolytic enzymes decreasing the total midgut protease activity. N-terminal amino acid sequencing of MChbL showed homology to osmotin-like protein from *Hevea brasilienisis* and α-amylase/trypsin inhibitor from *Zea mays* with 60% homology. The results show that MChbL may be useful in the development of insect resistance in important agricultural crops.

Keywords— Insecticidal activity, lepidoptera, mistletoe, *Viscum album.*

I. Introduction

LECTINS are among wide range of natural defense proteins found in plants [1]. They are heterogeneous group of proteins classified together on the basis of their ability to bind in a reversible way to well-defined simple sugars or complex carbohydrates. The main characteristic of these proteins is their ability to interact specifically with carbohydrates and to combine with glyco-components of the cell surface. While the physiological functions of plant lectins have not yet been fully elucidated, one possible function that of serving as a chemical defense towards large array of insect pests belong to the Coleoptera, Homoptera, Diptera and Lepidoptera order has been well documented [2]-[5]. Insecticidal activities of plant agglutinins are associated mostly with two main groups of plant lectins: monocot mannose-binding and chitin-binding lectin groups. GNA and wheat germ agglutinin are the best characterized representatives of these groups which are increasingly used in development of transgenic crops [6].

European mistletoe (*Viscum album* L.) is considered to be a toxic plant, and its content of toxic lectins lends support to this. Poison centers report toxicity of the whole plant, but especially the berries [7]. Due to the environmental concerns of pesticide use and limited list of effective alternatives mistletoe lectins would be promising candidates for biological pesticides that have low mammalian and environmental toxicity.

In the present paper we characterized *Viscum album* agglutinin (MChbL) and showed its antinutritive effect towards serious herbivore Lepidoptera pests of agricultural importance *Apamea sordens* Hufn. and *Agrotis segetum* Schiff. (Lepidoptera: Noctuidae). We found chitin-binding mistletoe lectin has high homology to thaumatin protein family and demonstrated its detrimental effect on larval development at different growth stages.

The possible implication of mistletoe agglutinin as biological pesticide against Lepidoptera pests is proposed.

II. Materials and Methods

A. Materials

The fruits of European Mistletoe were harvested in mountainous region of East-Georgia, in winter (December-February) and stored at -15°C until use.

B. MChbL Purification

Mistletoe chitin-binding lectin (MChbL) was prepared as described with some modifications [8]. The plant material was homogenized in medium consisting of 0.05 M Na-acetate buffer, pH 4.5, at ratio 1:3 (g/ml). The extracts were centrifuged at 5,000g for 15 min; supernatant was filtered through Miracloth (Calbiochem., USA) and Watman GF/c filter. The soluble protein fractions were purified by affinity chromatography on the agarose (Serva) and chitin (Sigma) sorbents, dialyzed, lyophilized and stored until use.

C. Insect Feeding Trials

The larvae of *A. sordens* and *A. segetum* were obtained from Khashuri region (East Georgia). Larval cultures were reared continuously at 25±1°C and relative humidity of 65-75%, under a L16/D8 light regime. To examine the effects of MChbL on insect larvae, they were maintained in plastic boxes, with perforated plastic covers and reared on a control and experimental diet with or without lectin, respectively. The lectins were incorporated into natural diet daily at a level of 0,001% (w/w). 10-15 larvae were used per treatment. Insect survival was estimated daily, the weights of larvae and pupae were measured and the duration of developmental

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stages was determined. The effect of MChbL on the development was assessed by determining the number and mass of surviving larvae.

D. Gut Enzyme Assays

Total gut protease activity was measured by FITC-casein assay. Fluorescein isothiocyanate was purchased from Sigma Chemical Co (USA). FITC labeled casein was prepared as follows: casein (10 mg) and FITC (4 mg) were dissolved in 2 mL of 0.1M sodium carbonate buffer (pH 9.0) containing 8M urea and left for 3 h at 20°C. FITC labeled casein was separated by gel chromatography on a Sephadex G-25 column (10 mL) equilibrated with 10 mM phosphate buffered saline (pH 7.5) (PBS); the visible FITC-casein fraction was pooled, desalted by dialyzing against distilled water, and lyophilized.

Midguts were isolated by dissecting the fifth instar larvae. The gut tissue was mixed with 3 volumes of 0,1M Gly-NaOH buffer (pH10.0) and allowed to stand for 15 min on ice to extract proteases. The gut luminal contents were recovered by centrifugation at 10,000g for 10 min at 4°C. The resulting supernatant was analyzed for protease assays. MChbL was preincubated with gut extract at 37°C for 15 min, prior to addition of the substrate. The enzyme solution (20 µl) was added to 40 µl of FITC-casein (1 µg/ml, in 0.1M Gly-NaOH buffer (pH10.0) and incubated at 37°C for 1 h. The reaction was stopped by adding 5 µl of 60 % trichloroacetic acid (TCA). The solution was mixed with 200 µl of 0.2M Tris-HCl buffer (pH9.0) containing 0,5% SDS and 0,02% NaN_3. The fluorescence polarization of samples was measured with Ex: 490 nm and Em: 520 nm. Each assay was carried out in triplicate.

Trypsin Inhibitor Activity was determined by a continuous rate spectrophotometric assay and expressed as the inhibition of BAEE units. Soybean trypsin inhibitor from *Glycine max* (soybean) was used as standard.

E. Amino Acid Sequencing

For amino acid sequence of NH_2-terminal domains, purified proteins were transferred electrophoretically to Trans-Blot PVDF membrane (BIO-RAD) by the method of Laurriere [9]. PVDF membrane was stained with 0.1 % Coommasie Blue R-250. The protein bands were cut out, destined and subjected to N-terminal sequencing on a protein sequencer (PPSQ-10, Shimadzu, Japan). Homologous sequences were searched by FASTA program.

III. Results And Discussion

The effects of MChbL on surviving of the larvae at different developmental stages is shown in Fig.1. Generally, in experimental groups the larval mortality observed were higher then that of control group. The mortality of third and fourth instar larvae fed on MChbL were 36% and 50%, respectively, compare to that of control insects (83%). The results suggest that the influence of lectins were much evident at the early stages of larval development. Apparently, this is related to the more sensitivity of glycosylated gut structures of young insects to carbohydrate-binding plant lectins. At the following stages of development lectin did not show significant influence on *A. sordens* larvae survival. Supposedly, larvae are less susceptible to deleterious effects of lectins at their late developmental stages. Pupae period and pupae weight were not significantly different among each treatment of both insects.

In the following series of experiments larvae midgut enzyme extracts were prepared and inhibitory effects of MChbL on midgut proteases activity were determined. Proteolytic activity of the midgut extracts from fifth instar larvae was measured by fluorescence polarization spectroscopy using FITC-labeled casein as substrate. The results showed that MChbL influenced larval gut proteolytic enzymes activity (decrease of total protease activity of the midgut extracts was monitored). The highest inhibition was 60% at a concentration of 0.25µg/µl MChbL (Fig.2).

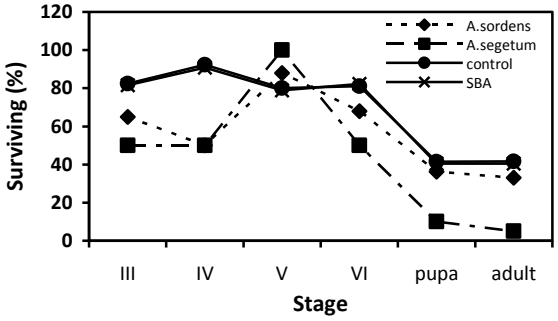

Fig.1 Effect of MChbL on the survival and development of *A.sordens* and *A.segetum* when incorporated into an artificial diet at 0,001% (w/w). Insects were newly emerged third instars larvae at the start of the assay.

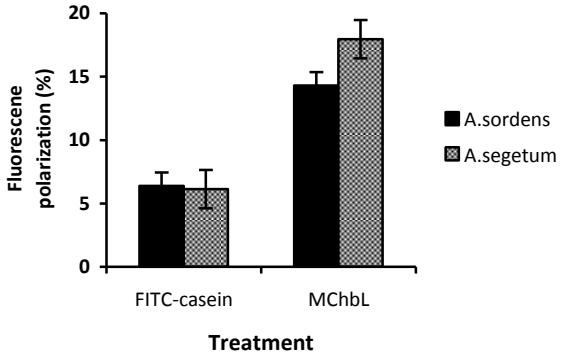

Fig.2 Effect of the MChbL on the proteolytic activity of midgut extracts from *A.sordens* and *A.segetum*. FITC-casein: substrate solution of FITC-casein was added to *A.sordens* and *A.segetum* midgut extracts; MChbL: *A.sordens* and *A.segetum* midgut extracts were preincubated with MChbL (ANOVA, n=20, $p<0.05$; Student's test).

When incubated with the insect enzymes MChbL showed resistance to digestion and no inhibition of sugar-binding activity of lectin was observed. Resistance to degradation by pest metabolic systems is clearly beneficial for plant defensive proteins, production of which represents an effective strategy developed by some plants [10].

MChbL showed no trypsin inhibitory activity towards bovin trypsin (data not shown), indicating the possible digestibility of MChbL by mammalian gut enzymes.

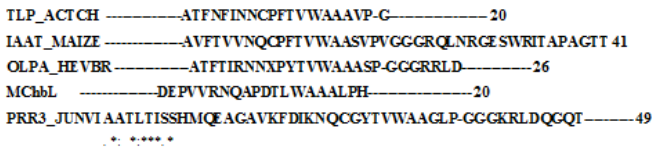

Fig.3 Aligned amino acid sequences of MChbL and homologous proteins. The identical amino acids are starred. MChbL, chitin-binding lectin from V.album; TLP_ACTCH, thaumatin-like protein from Actinidia chinensis; IAAT_MAIZE, alfa-amylase/trypsin inhibitor from Zea mays; OLPA_HEVBR, osmotin-like protein from Hevea brasilienses; PRR3_JUNVI, pathogenesis-related protein (PR) from Juniperus virginiana.

The N-terminal domains of MChbL were determined by applying of carboxamido-methylated proteins to sequential Edman degradation. The first 20 amino acids in N-terminal region of MChbL 23 kDa polypeptides showed the following sequence: Asp-Glu-Pro-Val-Val-Arg-Asp-Gln-Ala-Pro-Asp-Thr-Leu-Trp-Ala-Ala-Ala-Lys-Pro-His. Homologous sequences searched by FASTA program revealed high homology between NH_2-terminal domains of MChbL and deduced amino acid sequences of thaumatin protein family: osmotin-like protein from *Hevea brasilienisis* and α-amylase/trypsin inhibitor from *Zea mays* with 60% homoloy, thaumatin-like protein from *Actinidia chinensis* (Kiwi plant) with 61.5% homoloy and pathogenesis-related protien from *Juniperus virginiana* with 53.3% homology (Fig.3). In contrary, no sequence homologies were found between MChbL and plant toxins like RIPs or other mistletoe chitin-binding ViscalbCBA and cbML lectins. Thaumatin-like proteins belong to pathogenesis-related (PR) protein group expressed in the plants upon elicitor induction. Despite the structural divergence with thionins and hevein-containing other chitinases, high homology with α-amylase/trypsin inhibitor from *Zea mays* is the prerequisite for the entomotoxicity of proteins, therefore responsible for the plant defense against herbivorous pests.

The results obtained demonstrate that mistletoe chitin-binding lectin has obvious anti-nutritive effects on Lepidoptera larvae. Apparently, lectin exerts its antinutritive effect at early stages of development by interaction with midgut structures. The precise mechanism how the lectin exerts the insecticidal activity has not been fully elucidated. However, since glycoproteins are the major constituents of insect gut structures, it is possible that specific interaction take place between the glycosylated gut structures and plant lectins [11]. It appears, that surviving the hostile proteolytic environment of the insect midgut, specific binding to insect gut chitin components and alteration of glycosylated enzymes of digestive tract are basic prerequisites for MChbL lectin to exert its deleterious effects on insects. The insecticidal activity of MChbL may be attributed to the lectin-induced reduction in diet ingestion resulting starvation of larvae. Possible implication of mistletoe chitin-binding lectin as potential entomotoxic biopesticide for the control of polyphagous herbivore Lepidoptera pests is considered.

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