Isolation and characterization of venom proteins from the endoparasitoid wasp *Cotesia rubecula*

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## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Declaration</td>
<td>viii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>ix</td>
</tr>
<tr>
<td>List of publications</td>
<td>x</td>
</tr>
<tr>
<td>List of figures and tables</td>
<td>xi</td>
</tr>
<tr>
<td>Abstract</td>
<td>xiv</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>xvii</td>
</tr>
<tr>
<td><strong>Chapter 1: Literature review</strong></td>
<td></td>
</tr>
<tr>
<td>1.1 Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Insect immune system</td>
<td>3</td>
</tr>
<tr>
<td>1.2.1 Recognition molecules</td>
<td>4</td>
</tr>
<tr>
<td>1.2.2 Cellular responses</td>
<td>5</td>
</tr>
<tr>
<td>1.2.2.1 Phagocytosis</td>
<td>6</td>
</tr>
<tr>
<td>1.2.2.2 Encapsulation</td>
<td>6</td>
</tr>
<tr>
<td>1.2.2.3 Nodule formation</td>
<td>8</td>
</tr>
<tr>
<td>1.2.3 Humoral responses</td>
<td>8</td>
</tr>
<tr>
<td>1.3 Immune evasion by parasitoids</td>
<td>10</td>
</tr>
<tr>
<td>1.3.1 Passive mechanisms of immune evasion</td>
<td>10</td>
</tr>
<tr>
<td>1.3.2 Active mechanisms of immune evasion</td>
<td>11</td>
</tr>
<tr>
<td>1.4 Factors mediating immune suppression</td>
<td>12</td>
</tr>
<tr>
<td>1.4.1 Ovarian proteins</td>
<td>12</td>
</tr>
<tr>
<td>1.4.2 Polynaviruses</td>
<td>13</td>
</tr>
<tr>
<td>1.4.2.1 Polynavirus-mediated changes in cellular responses</td>
<td>16</td>
</tr>
<tr>
<td>1.4.2.2 Polynavirus-mediated changes in humoral responses</td>
<td>16</td>
</tr>
<tr>
<td>1.4.2.3 Polynavirus-mediated changes in host development</td>
<td>17</td>
</tr>
<tr>
<td>1.4.3 Venom proteins</td>
<td>18</td>
</tr>
<tr>
<td>1.4.3.1 Ectoparasitoid venom proteins</td>
<td>18</td>
</tr>
<tr>
<td>1.4.3.2 Endoparasitoid venom proteins</td>
<td>21</td>
</tr>
</tbody>
</table>
1.5 The endoparasitoid wasp *C. rubecula*

1.5.1 Crp32

1.5.2 CrBVs

1.5.3 venom proteins

1.6 Summary and program aims

**Chapter 2: General materials and methods**

2.1 Introduction

2.2 Insect culture

2.2.1 *Pieris rapae* culture

2.2.2 *Cotesia rubecula* culture

2.3 *P. rapae* cell line culture

2.4 Isolation of fat body, hemocytes and hemolymph

2.5 Isolation of polydnaviruses

2.6 Extraction of DNA from tissues or cells

2.7 Preparation of plasmid DNA

2.8 Extraction of DNA from gels

2.9 Extraction of total RNA

2.10 Construction of a *C. rubecula* ovary/venom cDNA library

2.10.1 Total ovary/venom RNA extraction

2.10.2 SMART cDNA synthesis by primer extension

2.10.3 Construction of cDNA library

2.11 Screening of libraries with anti-venom antibodies

2.11.1 Screening library to isolate single positives

2.11.2 Converting λTriplEx2 to plasmid TriplEx2

2.12 Screening of libraries with DNA probes

2.13 Determination of DNA and RNA concentration

2.14 DNA sequencing and sequence analysis

2.15 Southern blot analysis
2.15.1 Transfer of DNA from gel to membrane
2.15.2 Pre-hybridisation and hybridisation
2.15.3 Washing and autoradiography
2.16 Northern blot analysis
2.17 DNA and RNA slot blots
2.18 Removal of hybridized probe from membrane to allow reprobing
2.19 Reverse transcription-PCR (RT-PCR)
2.20 Preparation of transformation-competent cells
2.21 Heat-shock transformation of competent *E. coli* cells
2.22 rpHPLC and mass spectrometry
2.23 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)
2.24 Coomassie staining and destaining of polyacrylamide gel
2.25 Western blotting
2.26 Expression and purification of fusion proteins in *E. coli*
2.27 Preparation of antibodies
2.28 Elution of antibodies
2.29 Henalymph prophenoloxidase enzyme activity assay
2.30 Concentrations of proteins using microassays with microtiter plates
2.31 Vectos, markers, antisera and proteins
2.32 Solutions and media

Chapter 3: Isolation and characterisation of Vn4.6

3.1 Introduction

3.2 Materials and methods
3.2.1 Reverse phase HPLC and mass spectrometry
3.2.2 Peptide sequencing
3.2.3 RNA isolation and RT-PCR
3.2.4 Screening a venom-specific cDNA library
3.2.5 Enzymatic assays
3.2.6 Hemolymph phenoloxidase enzyme activity

3.3 Results and discussion

3.3.1 Purification of venom proteins
3.3.2 Sequence analysis of Vn4.6
3.3.3 Expression of Vn4.6
3.3.4 Enzyme inhibition assays

Chapter 4: Isolation and characterisation of Vn50

4.1 Introduction

4.2 Materials and methods

4.2.1 Insects and isolation of hemolymph and hemocytes
4.2.2 Reverse phase HPLC (rpHPLC)
4.2.3 N-terminal and peptide sequencing
4.2.4 RNA isolation and RT-PCR
4.2.5 Screening a cDNA library
4.2.6 Production and purification of Vn50 expressed in E. coli
4.2.7 Production of anti-Vn50 antibodies
4.2.8 Detection of glycosylation
4.2.9 Enzymatic assays
4.2.10 Hemolymph phenoloxidase enzyme activity
4.2.11 Stability of Vn50 in P. rapae hemolymph
4.2.12 Identification of hemolymph proteins bound to Vn50
4.2.13 Quantification of Vn50 injected into P. rapae larvae

4.3 Results

4.3.1 Isolation and characterisation of Vn50
4.3.2 Enzymatic assays
4.3.3 Inhibition of phenoloxidase activation
4.3.4 Status of Vn50 in the host hemolymph following parasitization
4.3.5 Identification of plasma proteins bound to Vn50
Chapter 5: Isolation and characterisation of calreticulin

5.1 Introduction

5.2 Materials and methods
  5.2.1 Insects
  5.2.2 Screening a cDNA library using anti-venom antibodies
  5.2.3 Recombinant protein expression and purification
  5.2.4 Production of a specific antibody
  5.2.5 SDS-PAGE and Western blotting analyses
  5.2.6 Isolation of RNA and S10 blotting
  5.2.7 Inhibition of hemocyte aggregation and spreading
  5.2.8 In vitro encapsulation assay
  5.2.9 Early-stage encapsulation-relasing protein assays

5.3 Results
  5.3.1 Isolation and characterisation of calreticulin
  5.3.2 Production of recombinant CrCRT in E. coli
  5.3.3 Tissue-specific localization of CrCRT
  5.3.4 Inhibition of hemocyte aggregation and spreading by CrCRT
  5.3.5 Effect of CrCRT on encapsulation in vitro
  5.3.6 Identification of an early-stage encapsulation-relasing protein

5.4 Discussion

Chapter 6: A novel venom peptide is required for expression of polydnavirus genes in host hemocytes

6.1 Introduction

6.2 Materials and methods
  6.2.1 Insect culture
  6.2.2 Bracovirus isolation
6.2.3 Reverse phase RP LC and mass spectrometry
6.2.4 Peptide sequencing
6.2.5 Peptide Vn1.5 synthesis
6.2.6 CrBV infection of hemocytes \textit{in vitro}
6.2.7 DNA and RNA isolation
6.2.8 Reverse transcription-PCR (RT-PCR)
6.2.9 DNA and RNA slot blot
6.2.10 Hemocyte changes after CrBV infection with or without Vn1.5
6.2.11 Transmission electron microscopy

6.3 Results
6.3.1 Venom is required for CrBV gene expression
6.3.2 RP-HPLC separation of venom proteins and bioassay
6.3.3 Mass spectrometry and peptide sequence analyses
6.3.4 Effect of synthetic peptide Vn1.5 on the CrBV gene expression
6.3.5 Hemocyte changes after CrBV incubation with or without venom
6.3.6 Observation by electron microscopy

6.4 Discussion

Chapter 7: Factors influencing the entry of CrBVs into host cells
7.1 Introduction
7.2 Materials and methods
7.2.1 Insect cultures and virus purification
7.2.2 Cell line culture
7.2.3 Virus uptake assays
7.2.4 Effect of temperature on CrBV entry into cells
7.2.5 Effect of pH on CrBV entry into cells
7.2.6 Treatment of cells with rinosomotropic agent
7.2.7 Effect of venom proteins on CrBV entry into cells
7.2.8 Total DNA isolation from infected cells and slot blot assays
7.3 Results

7.3.1 Assay for CrBV uptake into cells 145
7.3.2 Effect of temperature on CrBV uptake 146
7.3.3 CrBV uptake at different pH values 146
7.3.4 Lysosemotropc agent NH4Cl inhibits CrBV uptake 146
7.3.5 Venom proteins are not essential for CrBV entry into cells 147

7.4 Discussion 147

Chapter 8: General discussion 155

References 163
Abstract

Endoparasitoid wasps oviposit and develop in the hemocoel of their host where they are exposed to the host immune system, including humoral and cellular responses. Endoparasitoids have necessarily evolved effective mechanisms through which they inhibit the immune responses for successful parasitism. Inhibition of insect immune responses may be associated with changes in hemocyte population, down-regulated phenoloxidase activity, and aberrant hemocyte morphologies. To that end, maternal factors are introduced into host together with eggs during oviposition. These factors include symbiotic polydnaviruses, talyx proteins and venom proteins, and may act alone or together with one or more of the other factors to suppress the host immune responses.

Coesia rubecula (Hym: Braconidae) is an endoparasitoid of Pieris rapae (Lepidoptera: Pieridae), a worldwide insect pest of cruciferous crops. During oviposition, C. rubecula introduces maternal factors into the host, which facilitate successful parasitisation. In this study, several major venom proteins from C. rubecula were isolated and characterized including Vn1.5, Vn4.6, Vn50 and Carecticulin (CRT). Vn1.5 is a small novel peptide containing 14 amino acids with a molecular mass of 1598 Da. No similar sequences were found in protein databases. Although Vn1.5 is not essential for CrBV entry into host cells, it is required for CrBV gene expression in host hemocytes. In the absence of Vn1.5, CrBV transcription was not detected and hemocyte behaviour was not changed.

Vn4.6 has an open reading frame (ORF) of 129 nucleotides encoding 42 amino acids with a molecular mass of 4.6 kDa. The coding region for Vn4.6 is located upstream in opposite direction of a gene coding for a C. rubecula PDV-protein, Crp32. Transcripts corresponding to Vn4.6 were detected only in the venom gland and Crp32 only in the ovary (Asgari et al., 1998). Sequence similarity searches using PRINTS blast search showed that Vn4.6 protein has 25.5% identity with the neurotoxin α-atacotoxin-HV1A from the Australian funnel web spider Hadronyche versuta. Vn4.6 interferes with the activation of the host hemolymph prophenoloxidase (proPO), but not completely. This inactivation might have synergistic effects in conjunction with Vn50 (see below) and the
immuno-suppressive action of polydnaviruses introduced by the female into the host hemocoei.

The complete open reading frame of Vn50 contains 1167 nucleotides encoding 388 amino acids with molecular mass of 50 kDa. Vn50 is heavily glycosylated. Sequence homology searches at GenBank showed that Vn50 has high similarity to serine proteinase homologs (SPHs). These proteins, including Vn50, do not have proteolytic activity since the serine at the active site of the proteinase-like domain is changed to glycine. Vn50 sequence contains all the cysteine residues conserved in amino-terminal clip and serine proteinase domains of SPHs. Vn50 is stable in the host hemolymph – it remained intact for at least 72 h after being introduced into the host. Results show that Vn50 did not inhibit active phenoloxidase (PO) or proPO-activating proteinase, but it significantly reduced the proteolysis of proPO. This pathway might be employed by this endoparasitoid to negatively impact the activation in its host to suppress the host immune responses.

*C. rubecula* calreticulin (CrCRT) has been isolated from both polydnaviral particles and venom gland. The open reading frame of CrCRT contains 1209 nucleotides coding for 403 amino acids. At the end of the C-domain, a conserved HDEL motif was identified. Sequence alignment in GenBank showed a high similarity between the gene product of the isolated cDNA and CRTs. The highest similarities were found with *Galleria mellonella* (73.8%) and *Anopheles gambiae* (69.2%) CRTs. The predicted size for CrCRT is 46.5 kDa with a pI of 4.40, although CRT usually runs atypically at 60 kDa on SDS-PAGE. Slot blot analysis also showed that CrCRT is highly expressed in the venom glands and ovaries compared to the rest of the body. This protein can inhibit hemocyte aggregation and spreading, and this inhibition is dose-dependent. It also protects abiotic objects against host encapsulation response. Since host-specific CRT was identified from the host, this suggests that the soluble parasite-specific CrCRT might function as an antagonist molecule, competing for the binding site(s) with the host CRT.

Moreover, factors influencing the entry of CrBV into host hemocytes were investigated. The results showed that CrBV most likely initiate uptake at low pH in a pathway analogous to that of pH-dependent viruses. Venom proteins are not essential
for CrBV entry into host hemocytes, although they are required for CrBV gene expression in host hemocytes. Venom proteins from this wasp do not have antibacterial activity (data not shown).