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Genomic and peptidomic analyses of the neuropeptides from the emerging pest, *Drosophila suzukii*

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ABSTRACT

Drosophila suzukii is a highly polyphagous invasive pest which has been recently introduced into Europe and North America, where it is causing severe economic losses through larval infestations of stone and berry fruits. The peptidome of the selected nervous tissues of adult *D. suzukii* was investigated as a first step in identifying potential targets for the development of novel insecticides. Through *in silico* analyses of the *D. suzukii* genome databases 28 neuropeptide families, comprising more than 70 predicted peptides were identified. Using a combination of liquid chromatography and mass spectrometry of tissue extracts, 33 predicted peptides, representing 15 different peptide families were identified by their molecular masses and a total of 17 peptide sequences were confirmed by ion fragmentation. A comparison between the peptides and precursors of *D. suzukii* and *D. melanogaster* shows they are highly conserved, with differences only identified in the amino acid sequences of the peptides encoded in the FMRFamide, hugin and ecdysis triggering hormone precursors. All other peptides predicted and identified from *D. suzukii* appear to be identical to those previously characterized from *D. melanogaster*. Adipokinetic hormone was only identified in the corpus cardiacum, other peptides present included short neuropeptide F, a pyrokinin and myosuppressin, the latter of which was the only peptide identified from the crop nerve bundle. Peptides present in extracts of the brain and/or thoracico-abdominal ganglion included allatostatins, cardioacceleratory peptide 2b, corazonin, extended FMRFamides, pyrokinins, myoinihibitory peptides, neuropeptide-like precursor 1, SIFamide, short neuropeptide F, kinin, sulfakinins and tachykinin related peptides.

Introduction

Drosophila suzukii Matsumura, is a vinegar fly native to Asia. It is a highly polyphagous invasive pest which has been recently introduced into Europe and North America, where it is commonly known as the spotted winged *Drosophila* [14].

In contrast to most other fruit flies which infest overripe or decaying fruit, a prominent serrated ovipositor permits female *D. suzukii* to penetrate and lay eggs in unripe fruit (predominantly berry and stone fruit). This results in severe economic losses through larval infestations and damage, often leading to secondary infection by pathogens [18,54].

Since its reported introduction into Europe and North America in 2008, the biology, ecology and management of this invasive pest has received much attention [18,54], but knowledge in other areas, such as its behavior and physiology, is lacking. However, the recent publication of the genome and transcriptome of *D. suzukii* from an Italian alpine population [41] and the sequencing and annotation of the genome from a North American strain [15] will facilitate genomic and functional studies. These will yield insights into the evolution and adaptation of this pest as well as comparative analyses with other *Drosophila* and insect species. This genomic data also contains the information of all proteins and peptides (as gene precursors) and hence virtually all biochemical and physiological processes that occur, which will aid in the identification of new insecticide targets.

Neuropeptides and their cognate receptors, which have a central role in the regulation of physiological and behavioral processes in insects, are considered important targets for the development of novel pesticides [26,49]. A variety of insect neuropeptides and neuropeptide analogs have been shown to be insecticidal. These include the PISCF allatostatins (ASTs) and insect kinins which, when fed to aphids, cause significant mortality [23,38]. Others such as FGLa/ASTs and short neuropeptide F have been shown to regulate

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feeding and foraging behavior [33,55]. Furthermore, the down regulation of G protein-coupled receptors involved in larval growth, molting and metamorphosis in *Tribolium castaneum* were found to be lethal, thus identifying targets for novel pesticides [11].

There are numerous reviews and reports on the neuropeptides, peptide and protein hormones, and their receptors from insects. This is particularly evident for *Drosophila melanogaster* [28,39,50,53], the first insect for which its genome was sequenced and annotated [2]. Other insect genomes have since been sequenced, and in June 2011 the i5k Initiative was launched aimed at sequencing the genomes of 5000 insects and other arthropods that are considered to be important for agriculture, medicine, food security and energy production [1]. The availability of sequenced and annotated insect genomes has considerably assisted the identification of peptides and proteins from insect tissues by mass spectrometric techniques. Moreover, proteomics together with techniques such as functional genomics, RNA interference and targeted mutations, are providing information on essential physiological and behavioral processes that could be exploited for novel pest control strategies (reviewed by Boerjan et al. [13]).

As a first step in identifying insecticidal targets, the neuropeptide precursors from the *D. suzukii* genome databases and the corresponding peptides present in the central nervous system (CNS), have been investigated and identified.

Materials and methods

Genome analyses

To identify peptide sequences in the *D. suzukii* genome, the nucleotide sequences of *D. melanogaster* peptide precursors, acquired from flybase (http://flybase.org/) were used to search database whole-genome shotgun contigs using BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The annotated genome database spottedwingflybase (http://spottedwingflybase.oregonstate.edu) was also searched for peptide precursors. To identify the precursor for ion transport peptide, the open reading frame for *D. melanogaster* reported by Dircksen et al. [22] was used to search for the *D. suzukii* annotated transcript sequence.

Insects

D. suzukii were obtained from a culture maintained at the Food and Environment Research Agency, originating from Italy and were reared on *D. melanogaster* medium (Blades Biological Ltd, UK). Adults (1–2 weeks post-eclosion) of both sexes were used in this study.

Tissue extraction and liquid chromatography

One hundred brains were dissected from adult *D. suzukii* and placed in Eppendorf tubes containing ice-cold acidic methanol (87% methanol, 5% glacial acetic acid) and infused on ice for 30 min. The extraction medium was removed after centrifugation (4 °C, 12,000 × *g* for 20 min) and diluted 20-fold with 0.1% trifluoroacetic acid (TFA) for high performance liquid chromatography, performed using a Beckman System gold chromatography system (Beckman Coulter (UK) Ltd). The diluted sample was loaded onto a Jupiter C_{18} 10 μm 300 Å reversed-phase column (250 mm × 2.1 mm i.d.; Phenomenex, Macclesfield, UK). The column was eluted with a linear gradient of 5–60% acetonitrile/0.1% TFA over 55 min at a flow rate of 200 μl/min, and elution monitored at 214 nm. One minute (200 μl) fractions were collected and concentrated to *c*. 10 μl by centrifugal evaporation using a Savant Speed Vac concentrator (Thermo Electron, Basingstoke, UK) for mass analyses as previously described [3–5].

Single tissues of corpus cardiacum (CC), crop nerve bundle (CNB) and thoracico-abdominal ganglion (TAG) were dissected and analyzed directly (see below).

Mass and sequence analyses

Aliquots of HPLC fractions were mixed 1 μl:1 μl with matrix solution (α-Cyano-4-hydroxycinnamic acid; 10 mg/ml in 70% acetonitrile 0.1% TFA), 1 μl was then pipetted onto a MALDI sample plate and air dried. Dissected single tissues of the CC, CNB or TAG were transferred directly into 0.5 μl of HPLC-grade water on the MALDI sample plate. The water was immediately removed by blotting with filter paper and approximately 0.5 μl of methanol/matrix solution (1:1) added and allowed to dry.

Mass spectra were acquired using a Voyager DE STR MALDI TOF mass spectrometer (applied Biosystems, Warrington, UK) or a Bruker ultraflex mass spectrometer (Bruker Daltronic GmbH, Bremen, Germany) in positive reflectron mode over the mass range *m/z* 500–5000 Da [5]. Results are the mean of three independent MS measurements for each sample and all masses are shown as monoisotopic masses $[M+H]^+$.

External calibration was conducted using a calibration mixture containing des-Arg-bradykinin, angiotensin 1, Glu-fibrinopeptide B and neurotensin (Applied Biosystems) or angiotensin I, angiotensin II, substance P, bombesin, ACTH clip 1–17, ACTH clip 18–39, and somatostatin 28 (Bruker Daltronic).

Fragmentation of selected mass ions for sequence analyses was achieved on the Bruker ultraflex using LIFT™ technology and data was analyzed by FlexAnalysis software. Sequences of peptides were determined manually and/or by comparing the fragmentation patterns with those predicted for known and predicted peptides using Protein Prospector (University of California, San Francisco, USA).

Results

Genome data

Nucleotide Blast searches of the *D. suzukii* genome databases and gene queries of the annotated spottedwingflybase revealed the precursor sequences of 28 neuropeptide families, comprising more than 70 putative peptides (supplementary data Figure 1). Peptides and their calculated monoisotopic masses ($[M+H]^+$) were predicted from these precursors (Table 1).

Supplementary Figure 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.peptides.2014.08.006.

Mass and sequence analyses

The peptides with identical monoisotopic masses ($[M+H]^+$) to those of predicted peptides from precursor sequences determined by direct analyses of single CC, CNB or TAG tissues or of aliquots of HPLC fractions from an extract of 100 brains are shown in Table 1. Peptides of masses >5000 MW were not measured in this study, and masses >2500 Da did not correspond to any of the predicted peptides. Peptide sequences determined by fragmentation of the parent ions present in HPLC fractions of brain extracts are also identified in Table 1.

Single tissue analysis

Corpus cardiacum. A representative mass spectrum of a single CC is shown in Fig. 1A. The measured masses identical to the calculated monoisotopic masses of predicted *D. suzukii* peptides were identified.

The precursor for adipokinetic hormone (AKH) predicts an 8 amino acid peptide amidated at its C-terminus (QLTFSPDWamide).

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