Pestycydy, <u>2007</u>, (3-4), 39-43. ISSN 0208-8703

Novel Members of the AKH/RPCH Peptide Family: Isolation of AKH from the *Corpora Cardiaca* of the Two Beetle Species, *Cheilomenes Lunata* and *Coccinella Septempunctata*

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Abstract: Adipokinetic hormones (AKH) are synthesized in endocrine cells of the glandular *corpora cardiaca*, in the anterior part of the retrocerebral complex. AKH plays an important role in the metabolism of carbohydrates that fuel energy-depleting activities such as flight and walking. To date, the primary sequences of eight isoforms from various beetle species have been elucidated. In this study, the glandular part of the CC from two Ladybird beetles, *Cheilomenes lunata and Coccinella septempunctata* were directly analysed by MALDI-TOF mass spectrometry. Subsequently, the amino acid sequences of both putative novel AKHs were confirmed by tandem mass spectrometry.

Keywords: adipokinetic hormons (AKHs), MALDI-TOF mass spectrometry, insect, beetles

INTRODUCTION

Members of the adipokinetic hormon/red pigment-concentrating hormon family are exclusively produced in glandular cells of the retrocerebral complex which occupy, in most insects, the anterior part of the *corpora cardiaca* (CC). They are not synthesized in neurons of the nervous system, and thus, they are not neuropeptides [1]. The first AKH was isolated from the CC glands of locusts where it displays lipid-mobilising activity and fuel production for long-distance flight [2]. All AKHs are characterized by (1) N-terminally blocked pyroglutamate, (2) eight to ten amino acids, (3) at least two aromatic amino acids, as well as (4) overall mainly uncharged molecule [3, 4]. AKHs exert a wide range of diverse effects [4] such a regulation of carbohydrates and lipid metabolism, as well as, effectors for energy-costly activities [5, 6]. In beetles, like in other insects, proline is the amino acid that powers energy consuming activities as flight, walking and ball-rolling [6]. More than 30 different members of the AKH family were identified from various invertebrates [4].

In this study, the primary sequence of two putative novel AKHs from the European Ladybird beetle *Coccinella septempunctata* and the Southern African Ladybird beetle *Cheilomenes lunata* were identified by means of direct MALDI-TOF mass spectrometry. *De novo* sequencing yielded amino acid sequences similar to known AKH of *Tribolium castaneum* [7].

MATERIAL AND METHODS

Animals. Ladybird beetles, *Cheilomenes lunata* were caught in (...) Namibia by Dr. Reinhard Predel and *Coccinella septempunctata* were caught in Jena (Germany) by the author.

Sample preparation for mass spectrometry. The glandular area of the corpora cardiaca (CC) was dissected and prepared for MALDI-TOF mass spectrometric analysis as described by Predel [8].

MALDI-TOF mass spectrometry. Mass spectra in the mass range of 600-3000 Da were acquired in positive ion mode on a Bruker Daltonic ultraflexII TOF/TOF biospectrometry workstation (Bruker Daltonic GmbH, Bremen, Germany) equipped with a pulsed nitrogen laser. Laser strength was adjusted to provide an optimal signal-to-noise ratio. For the tandem MS experiments, the protonated PDF mass ion signal was selected and fragmented by tandem mass spectrometry (CID: collision-induced dissociation). The fragmentation data obtained in these experiments was handled using the Protein Prospector (http://prospector.ucsf.edu).

RESULTS AND DISCUSSION

Two novel octapeptide members of the AKH/RPCH family have been identified from the glandular part of the *corpora cardiaca* (CC) of two species of Ladybird beetles by means of direct MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometry (Figure 1.). The majority

of AKH have no charge which is represented by a characteristic ion signature $[M+Na]^+$ and $[M+K]^+$ in resulting mass spectra [8, 9]. Thus, the putative AKH of both beetle species are observed as $[M+Na]^+$: 1023.4 Da and $[M+K]^+$: 1039.4 Da. By using tandem mass spectrometry techniques, the same samples were subjected to CID (collision-induced dissociation). The manual analysis of the fragment series resulted in the sequence pQLNFTPNWamide, with a monoisotopic mass at $[M+H]^+$: 1001.4 Da (Table 1; Figure 2). Comparing with AKHs from other beetle species, the both ladybird beetle AKHs, designated as Chl-AKH (*Cheilomenes lunata*) and Cos-AKH (*Coccinella septempunctata*), shows sequence conformity with Trc-AKH of *Tribolium cataneum* (Table 1). An additional similarity of these three AKHs is an amino acid modification at position 5 (Thr) which could be of interest for phylogenetic studies.



Figure 1. MALDI-TOF mass spectra of CC-preparations of *Cheilomenes lunata* and *Coccinella septempunctata* in a mass range of 800-2000 Da. The arrow marks the ion signals of the putative AKH in both beetle species.



Figure 2. Representative CID spectrum of the ion signal at 1023.5 of *Cheilomenes lunata* which represents a Na⁺ alkali cation ion signal ([M+Na]⁺) of the putative Chl-AKH. A number of *y*- and *b*-type fragment ions are labelled. The fragments were analysed manually and the resulting sequence is given in the inset. The CID spectrum of Cos-AKH is identical and hence not shown.

Table 1.	Comparison of primary structure of peptides of the AKH/RPCH
	family in various beetle species. Similarities are bold labelled

Species	AKH-1		AKH-2		References
	peptide sequence	$[M+H]^{+}$	peptide	$[M+H]^{+}$	
		m/z	sequence	m/z	
Decapotoma lunata	pQLNFSPNWGNa	1158.53	pQLNFSPNWa	987.46	[10]
Onitis aygulus	pQYNFSTGWa	984.42	pQFNYSPDWa	1038.43	[11]
Gareta nitens	pQFNYSPVWa	1022.47	pQFNYSPDWa	1038.43	[12]
Scarabaeus deludens	pQFNYSPVWa	1022.47	pQFNYSPDWa	1038.43	[12]
Geotrupes stercorosus	pQLNYSPDWa	1004.44	-		[13]
Pachnoda marginata	pQLNYSPDWa	1004.44	-		[14]
Melolontha melolontha	pQLNYSPDWa	1004.45	-		[13]
Tenebrio molitor	pQLNFSPNWa	987.46	-		[15]
Zophobas rugipes	pQLNFSPNWa	987.47	-		[15]
Tribolium castaneum	pQLNFTPNWa	1001.48	-		[7]
Leptinotarsa decemlineata	pQLTFTPNWa	988.49	pQVNFSPNWa	973.52	[14]
Coccinella septempunctata	pQLNFTPNWa	1001.48	-		this study
Cheilomenes luncata	pQLNFTPNWa	1001.48	-		this study

Acknowledgments

I acknowledge the financial assistance of the Deutsche Forschungsgemeinschaft (PR 595/6-1, 2). I will also thank R. Predel (Jena) for critical discussions of the manuscript, and O. Scheibner (HKI, Department of Biostructure Chemistry Jena, Germany) for supporting tandem MALDI-TOF measurements.

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