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Amino Acid Sequence of an Adipokinetic Neuropeptide from the Plant Bug, *Iphita limbata* Stal

A.P. Ajaykumar and M. Gokuldas
Department of Zoology, Division of Insect Biochemistry and Physiology, University of Calicut, Kerala, 673635, India

Corresponding Author: A.P. Ajaykumar, Department of Zoology, Division of Insect Biochemistry and Physiology, University of Calicut, Kerala, 673635, India

ABSTRACT

Intermediary metabolism in insects is regulated by small neuropeptides of the adipokinetic hormone/red pigment concentrating hormone family (AKH/RPCH). AKHs are synthesized and stored in the endogenous neurosecretory cells of Corpora Cardiaca (CC). The present investigation was carried out to elucidate the primary structure of adipokinetic neuropeptides in the plant bug, *Iphita limbata* using HPLC, bioassay and mass spectrometric analyses (MALDI-MS and MS/MS). Separation of an extract of retrocerebral complexes of *I. limbata*, by RP-HPLC and monitoring at 210 nm showed six distinctive absorptive peaks. The chromatographic fractions were examined for adipokinetic activity by a homologous *in vivo* bioassay. Two UV absorbance peaks were found to contain compounds that are significantly active in mobilization of lipids from the fat body. Comparison of HPLC profile of synthetic peptide, Pyrap-AKH with that of the extract, showed that the peptide had identical retention time as that of material in the extract with significant hyperlipaemic effect. MALDI-MS analysis of the corpora cardiaca extract indicated that the molecular mass 1000.4 Da of the ion peak obtained is similar to that of Pyrap-AKH. MALDI-MS/MS analysis in PSD mode confirmed the primary structure of adipokinetic hormone of *I. limbata* is pE-L-N-F-T-P-N-W-NH_2 which is same as that of Pyrap-AKH. This data may be helpful for tracing the phylogeny of the insect order Heteroptera.

Key words: Adipokinetic neuropeptides, high performance liquid chromatography, matrix assisted laser desorption ionization mass spectrometry, *Iphita limbata*, amino acid

INTRODUCTION

The vertebrate hypothalamo-hypophysial system is well known to endocrinologists. Analogous structures are found in invertebrate groups: corpora cardiaca in the heads of insects and X-organ-sinus gland complex in the eye stalk of crustaceans. The hormones of X-organ-sinus gland complex are known to regulate reproduction, metabolism, osmoregulation, chromatic adaptation and growth (Shuranova *et al*., 2006; Velmurugan *et al*., 2008). Insect AKHs are also multifunctional (Kodrik *et al*., 2010). Neurosecretory materials control a variety of physiological functions. However, the isolation and structure identification of the active materials in these cells were successfully done only recently. Refinements in high-performance liquid chromatography and methods in peptide sequencing greatly contributed to this. Brown and Starratt (1975) for the first time identified an insect peptide called proctolin. The second insect neuropeptide to be identified was the adipokinetic hormone from the locust, *L. migratoria*

(Stone *et al*., 1976). It is now known that these peptides are members of a large family of structurally related peptides known under the acronym AKH/RPCH family. Common characteristics of the family are: a chain length of 8 to 10 amino acids; the N-terminus blocked by pyroglutamic acid (pGlu); the C-terminus blocked by a carboxyamide; amino acids at positions 8 and 9 (when present) are tryptophan and glycine and most of the peptides are uncharged. There are at least two aromatic amino acids, at position 4 mostly phenylalanine (but sometimes tyrosine) and at position 8 tryptophan and a few peptides have a third aromatic amino acid either at position 2 (Tyr or Phe) or at position 7 (Trp) (Gaede, 2006). The known actions of AKHs are, however, broader than what their name implies. The peptides have been reported from most of the insect orders. It acts on the fat body to mobilize stored lipids and carbohydrates, activate glycogen phosphorylase, accumulate cAMP (Goldsworthy, 1983; Ajaykumar and Gokuldas, 2011) and inhibit the synthesis of lipids (Gokuldas *et al*., 1988; Lorenz, 2001; Fabold *et al*., 2010), proteins (Carlisle and Loughton, 1979; Kodrik, 2008) and RNA (Kodrik and Goldsworthy, 1995). AKHs exhibit similarity with vertebrate hormone at functional level, AKHs resembles glucagon (Alquicer *et al*., 2009), a peptide hormone from the α-pancreatic islets cells in vertebrates and the vertebrate catecholamine adrenaline (Gaede, 2004). Another vertebrate candidate whose function can be compared with AKHs is vertebrate adiponectin, a hormone discovered relatively recently from vertebrate adipose tissue (Tsao *et al*., 2002) which increases the oxidation of fat and thereby reduces the intracellular triglyceride content of the liver and muscle and increases the cellular sensitivity to insulin (Diez and Iglesias, 2003).

The insect sub-order Heteroptera comprises the true bugs and forms the largest hemipteran group (Shcherbakov and Popov, 2002). Yet, to date, the AKHs have been isolated and identified only from fifteen bug species many of the important species are still awaiting such discoveries. The present investigation was carried out to elucidate the primary structure of adipokinetic neuropeptides in the flightless bug, *I. limbata* using HPLC, bioassay and mass spectrometric analyses.

MATERIALS AND METHODS

The data were obtained from the research work conducted in the Department of Zoology, University of Calicut, Kerala, India and Indian Institute of Science, Bangalore, India from July 2007 to April 2008.

Experimental insect: Adults of the plant bug, *I. limbata* were collected locally from the fields. A stock culture of insect was maintained in the laboratory on a diet of sprouted green gram (*Phaseolus aureus*) and ripe banana. The insects of both sexes were used for hormone extraction. Adult female insects, after keeping for at least one week in the insectary, were used for bioassays experiments.

Preparation extract of *Corpora cardiaca* and synthetic Pyrap-AKH: Adults of both sexes of the plant bug, *I. limbata* were used for collecting corpora cardiaca for hormone extraction. The retrocerebral complexes were removed with the help of a pair of fine forceps under a stereozoom binocular microscope (ZEISS, Germany). Tissue was immediately put into ice cold 80% methanol (HPLC grade) and stored at -4°C until extraction. Tissue was sonicated for 1 min on ice with an ultrasonicator (Sonics and Materials, USA). The extract was centrifuged at 4°C and 10,000 rpm for 10 min. The supernatant was collected into an eppendorf tube and vacuum dried (Savant, USA). The dried supernatant was stored at -4°C until used for further analyses.

Synthetic Pyrap-AKH was a gift from Dr. D. Kodrik, Institute of Entomology, Academy of Sciences, Brinisovsca, Czech Republic. The peptide (1.0 mg) was dissolved in 10 mL of 80% methanol (HPLC grade). Required concentration of the peptide solution was prepared from this stock solution.

HPLC analysis: The dried extract made from the retrocerebral complexes from *I. limbata* was dissolved in 20 μL of 80% methanol (HPLC grade). The solution was filtered using a sample filtration unit (Millipore, U.S.A) with 0.45 μm filter paper. The samples were directly injected into the instrument by a micro-syringe (Hamilton, 20 μL). HPLC separations were carried out using Shimadzu system (SPD 10 AVP, LC 10 ATVP, LC 10 ATVP) with a reversed phase column (C_{18}) 250 mm long, 4.6 mm i.d, in a binary gradient from 43 to 53% solvent B in 20 min with a flow rate of 1 mL min^{-1}. Trifluoroacetic Acid (TFA) 0.01% in water (HPLC grade) was used as solvent A, solvent B was 60% acetonitrile in solvent A. All the solvents were filtered through 0.45 μm filter paper. The eluants were monitored at 210 nm. The HPLC fractions 8 to 14 min were manually collected, freeze dried and used for bioassays of hyperlipaemic activities.

The presence of an already known AKH peptide, Pyrap-AKH in the retrocerebral extract of *I. limbata* was studied by HPLC analysis. Synthetic peptide Pyrap-AKH (200 pmol) was injected into the HPLC with same instrumental conditions used for extracts of retrocerebral complexes of *I. limbata*. The HPLC profiles of the two were overlaid for comparison.

Hyperlipaemic bioassays of fractions separated on HPLC: The hyperlipaemic activities of fractions of retrocerebral complex extract of *I. limbata* separated on HPLC were tested in female *I. limbata*. Each fraction was redissolved in 50 μL insect saline. Aliquots of 5 glands per equivalent (5 μL) were injected using a Hamilton microsyringe (10 μL) into the haemolymph of the insect. Haemolymph samples were collected before (control) and 60 min after (experimental) the injection and were used for quantitation of lipids.

Quantitation of haemolymph lipids: Total lipids in the haemolymph samples were determined using a modified phosphovanillin reagent (Frings *et al*., 1972) method. Haemolymph samples collected (2 μL each) collected in various experiments were deposited into the bottom of test tubes. Concentrated sulphuric acid (50 μL) were added to these samples, heated in a boiling water bath for 10 min, cooled to room temperature and 2 mL of each phosphovanillin reagent were added. The tubes were thoroughly shaken to mix the content. Optical densities were measured within 5 min using UV-vis spectrophotometer at 540 nm against a reagent blank.

MALDI-TOF-MS analysis: The dried extracts of neurohaemal tissues of the insect were used for mass spectrometric analysis. Mass spectrometric analysis were performed on an Ultra Flex mass spectrometer (Bruker Daltonics, Germany) in reflectron ion mode, using a 90 ns time delay and a 25 kV accelerating voltage in the positive ion mode. The extract was monitored in H^+ mode. The system utilized 50 Hz pulsed voltage laser, emitting at 337 nm. The ion source and the flight tube were kept at pressure of about 7×10^{-7} mbar by turbo molecular pump. The samples were prepared by mixing equal volumes of peptide solution and a saturated solution of matrix, dihydroxybenzoic acid in 1:1 (v/v) acetonitrile: water mixture. A standard peptide mixture was used for external calibration.

Tandem-MS/MS: Tandem Mass Spectra (MS/MS) were acquired by selecting the precursor mass (1001.4 Da in H^+mode) with a 10 Da window and fragments were generated in Post Source Decay (PSD) mode. A single acquisition was a sum of 360 added shots to generate the MS/MS spectra.

Mass spectra were analysed by using Flex-analysis software. The primary structure of the peptide was deduced by using Peptide Fragmentation Ion Analyser-II (PFIA-II) software (http://hodgkin.mbu.IIsc.ernet.in/~pfia/ PFIA-II.html).

RESULTS AND DISCUSSION

HPLC analysis and biological activity of peptide: The HPLC profile of retrocerebral extracts of *I. limbata* is provided in Fig. 1. The data showed six prominent absorption peaks (designated as 1, 2, 3, 4, 5 and 6) with retention times 7.2, 8.02, 8.85, 9.75, 12.11 and 13.48 min, respectively. Homologous *in vivo* bioassays were carried out to study the hyperlipaemic effects of materials present in the collected fractions (8-14 min) and the results are represented in Fig. 2. The materials in the 12 and 14 min HPLC fractions induced significant adipokinetic activities with increases by 65 and 45% ($p<0.05$) over control, respectively. The materials present in the other fractions (8, 9, 10, 11 and 13 min) also showed slight hyperlipaemic activities, viz., increases up to 10, 20,

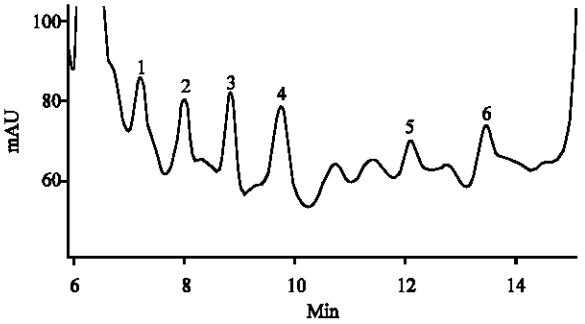

Fig. 1: HPLC profile of extracts of retrocerebral complexes of *I. limbata*. The analysis was carried out an a C_{18} Kibar column. The extract was run with a gradient of 43-53% B in 20 min (solvent = A = 0.01% trifluoro acetic acid in water, solvent B = 60% acetonitrile in solvent A). The eluants were monitored at 210 nm. Numbered are the major peaks

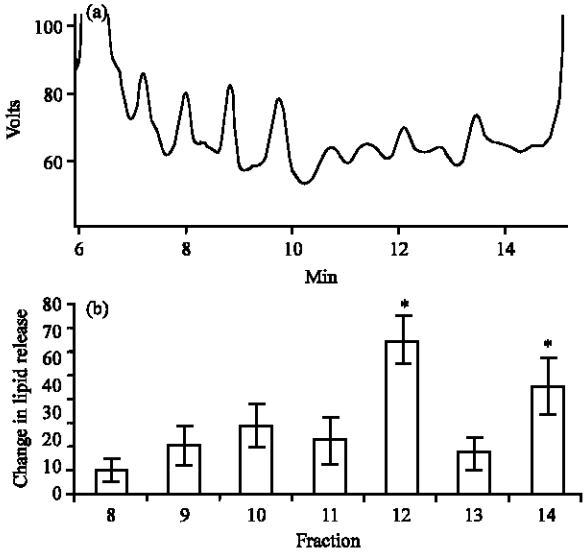

Fig. 2 (a-b): (a) HPLC profile of extracts of retrocerebral complexes of *I. limbata* and (b) peak fractions were collected, tested for hyperlipaemic activity. The change in total haemolymph lipid is reppresented, as E/C % in histogram. (*) Indicates $p<0.05$

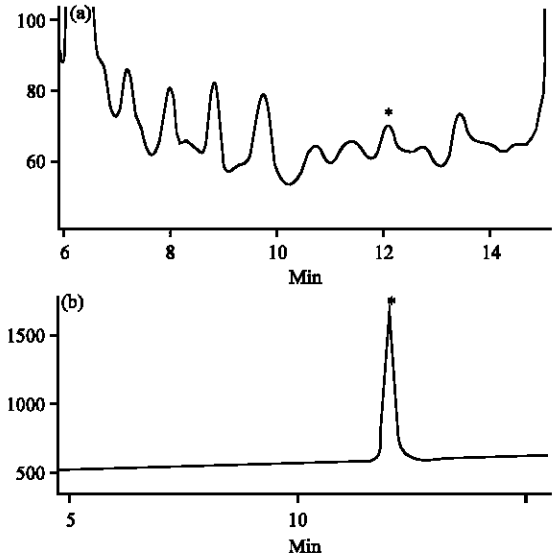

Fig. 3 (a-b): (a) HPLC profiles of crude cropra extract of *I. limbata* and (b) synthetic Pyrap-AKH, monitored at 210 nm. The analysis was carried out on a C_{18} Hibar column. The extract was run with a gradient of 43-53% B in 20 min (solvent A = 0.01% trifluoro acetic acid in water, solvent B = 60% acetonitrile in solvent A). The eluants were monitored at 210 nm. (*) Indicates peaks of interest

29, 22 and 16%, respectively which were, however, not statistically significant. The presence of Pyrap-AKH in the tissue extract was tested by injecting 200 pmol of the synthetic peptide into the HPLC instrument with identical instrumental conditions as before. The two chromatograms were compared (synthetic peptide and retrocerebral extract), the data showed that the active material in the fraction 12.11 min of the retrocerebral extract also had the same retention time as that of the synthetic Pyrap-AKH (Fig. 3).

Mass spectrometric analysis: The MALDI-MS spectra have given a few ion signals, among which two prominent hydrogen adducts $(M+H)^+$ of molecular ion peak with m z^{-1} value at 1001.4 was identified (Fig. 4), the peptide with mass 1000.4 Da was selected for further sequencing of the peptide in MALDI-PSD mode.

MALDI MS/MS of precursor peptide at m z^{-1} 1001.4 $(M+H)^+$: Fig. 5 represents the fragmentation pattern of the precursor ion 1001.4 Da $(M+H)^+$. The theoretical fragmentation pattern of Pyrap-AKH was deduced from web tool, PFIA-II. The identified C-terminal 'y' type ions are; y_3 (m z^{-1} 415.2) and y_6 (m z^{-1} 684.34). The N- terminal 'a' type ions are a_2 (m z^{-1} 197.12), a_4 (m z^{-1} 458.24), a_6 (m z^{-1} 656.34), a_7 (m z^{-1} 770.38) and a_8 (m z^{-1} 956.4) and 'b' type ions are; b_1 (m z^{-1} 112.03), b_3 (m z^{-1} 339.16), b_4 (m z^{-1} 486.2), b_5 (m z^{-1} 587.28), b_6 (m z^{-1} 684.33) and b_7 (m z^{-1} 798.37). From these informations, the primary structure of the precursor ion is derived as pE-L-N-F-T-P-N-W-NH_2 which is the same as that of the already known AKH/RPCH peptide Pyrap-AKH.

Earlier studies have demonstrated that the crude extracts of CC from *I. limbata* able to increase the levels of haemolymph lipid in homologous *in vitro* bioassays (Rasheed and Gokuldas, 2002). In the present study, HPLC fractions of the retrocerebral extracts were studied for their effect *in vivo*.

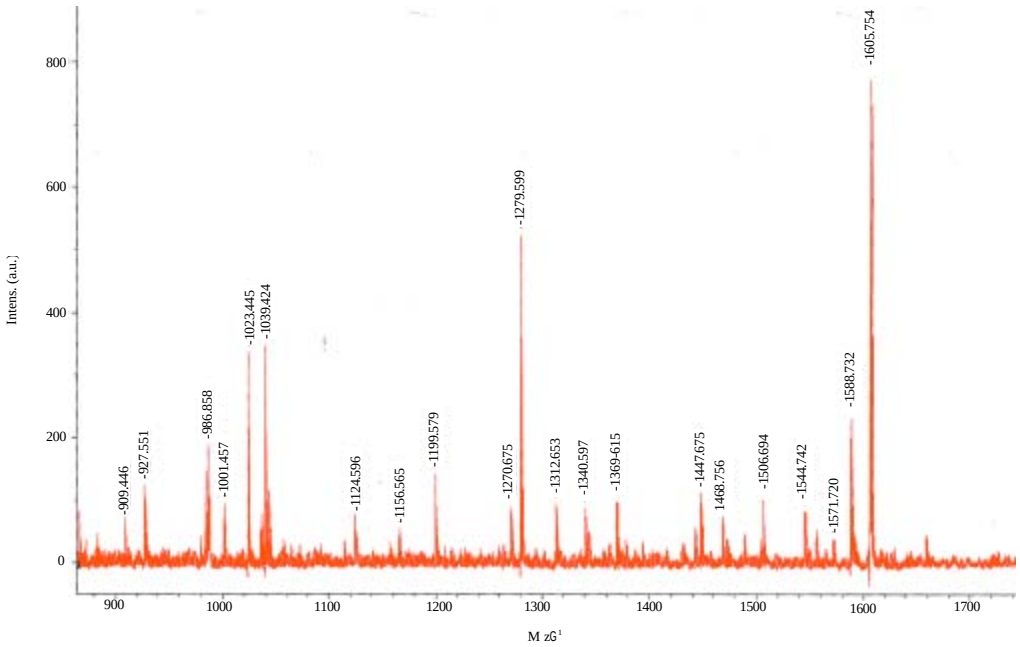

Fig. 4: MALDI-MS spectrum of extract of corpora cardiaca of *I. limbata*. The analysis was carried out in reflector positive $(H)^+$ mode with an accelaration voltage of 50 Hz pulsed N_2 laser, emitting at 337 nm. Dihydroxy benzoic acid was used as matrix

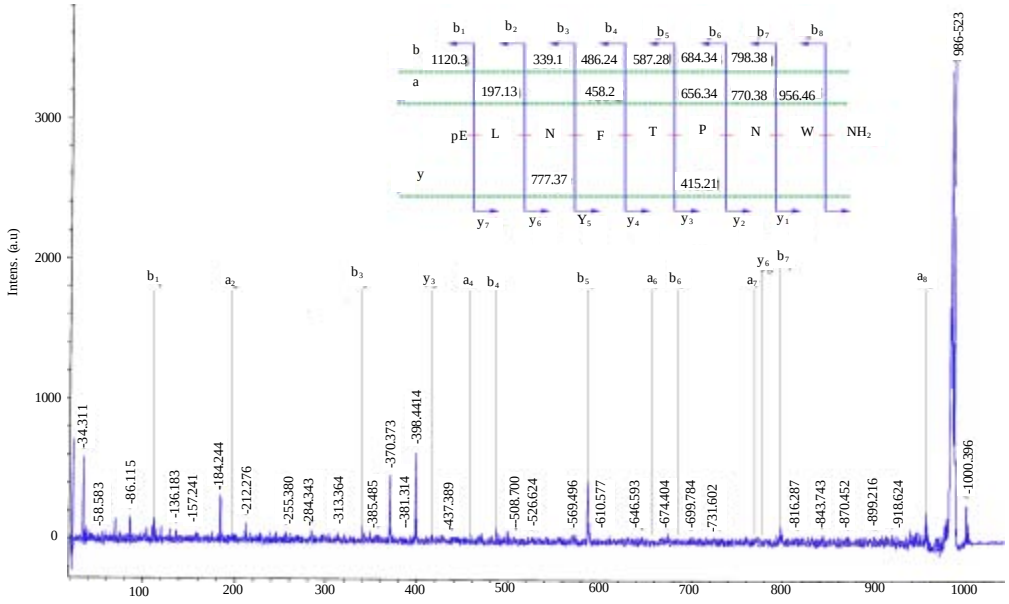

Fig. 5: MALDI-MS/SM spectrum of the ion $(M + H)^+ = 1000.4$ Da from *I. limbata*, inset shows the sequence assignment of the peptide, together with theoretical and calculated masses for "b", "y" and "a" type fragment ions, obtained in the MS/MS spectrum

It has been shown that some of these fractions have significant hyperlipaemic effects. In addition, the synthetic Pyrap-AKH had identical retention time as that of biologically active fractions of the tissue extracts separated by HPLC (Fig. 3) indicating similarity in the hydrophobic interaction of the active components in the retrocerebral complex extracts to that of Pyrap-AKH. The MALDI-MS and tandem MS/MS analysis confirmed the presence of already known Pyrap-AKH in *I. limbata*. These results are in agreement with the studies conducted by Kodrik *et al.* (2000). They demonstrated that the Pyrap-AKH elevated haemolymph lipid level in *P. apterus* during enhanced locomotor and dispersal activities even when flight was substituted by running (Socha *et al.*, 1999; Kodrik *et al.*, 2002a). Both, *P. apterus* and *I. limbata* come under the same family and they exhibit similarity in morphology, locomotion, feeding habits etc. Thus it is presumed that the Pyrap-AKH present in *I. limbata*may be helpful in locomotor activity by elevating haemolymph lipid level. Further studies are necessary to establish this proposition with respect to the locomotion in *I. limbata*.

Pyrap-AKH is the first heteropteran AKH/RPCH peptide isolated from the firebug, *Pyrrhocoris apterus*, (Family: Pyrrhocoridae) (Kodrik *et al.*, 2000). AKH/RPCH peptides have been isolated and identified from 15 bug species. The representative species of families Nepidae, Belostomatidae, Corixidae, Gerridae and Notonectidae of the same insect order Heteroptera were identified. Pyrap-AKH is an octapeptide, showing maximal sequence similarity with Locmi-AKH-I and Phymo-AKH-I. The first eight amino acid residues of Pyrap-AKH are identical to these peptides. Pyrap-AKH exhibited 87.5% similarity with five other octapeptides, Emppe-AKH, Locmi-AKH-III, Micvi-AKH, Peram-CAH-II and Tenmo-HrTH (indicating a change by one amino acid). It also exhibits structural similarity with four decapeptides (which means an exchange of one amino acid within the first eight amino acids of the molecule and the addition of two more amino acids at the C-terminus), Carmo-HrTH (*Carausius morosus* Hyper trehalosmic hormone), Phyle-CC (*Phymateus leprosis*), Declu-CC (*Decaptoma lunata*) and Rommi-CC (*Romalea microptera*). It has been observed that Pyrap-AKH has relatively low similarity (62.5%) with the cicada (*Platipura capensis*) peptides, Placa-HrTH-I and II (Kodrik *et al.*, 2000). In all crustacean species studied to date, there is only one RPCH which is identical in all arthropod species. In contrast, most of insect orders have more than one AKH peptides with amino acid substitution or difference in chain (Marco, 2004) Recently RPCH was isolated and sequenced from a heteropteran insect, viz. stinkbug *Nezara virudula*, in which it mobilize lipids (Gaede *et al.*, 2003). Thus conservation of peptide structure and functional cross reactivity of RPCH and AKH across two phylogenetic groups have been demonstrated. The information on the AKH/RPCH family indicates that genes and preprohormone processing mechanism have been conserved during evolution of insects and crustaceans.

Most phylogenetic relationships of organisms are mainly based on morphological data and many of the phylogenetic relationships of invertebrate species are still under dispute. Analysis of primary structures of the AKH peptides has shown that there exists an order or family specificity. Such data have been used as additional information to aid in the construction of phylogenetic trees by means of computer programme and protein parsimony algorithms (Gaede and Marco, 2009). Considering the Heteropteran family Pyrrhocoridae, the results of present study do not contradict but rather support the relationship among the species of this group. Yet to date, AKHs have been isolated and identified from three species of the family Pyrrhocoridae, *Pyrrhocoris apterus* (Kodrik *et al.*, 2000), *Dysdercus intermedius* (Kodrik *et al.*, 2010) and *Cenaeus carnifex* (Socha *et al.*, 2004) of them posses Pyrap-AKH. Recent observations of Gaede and workers

Table 1: Heteropteran species having the adipokinetic neuropeptide, Pyrap-AKH in their *Corpora cardiaca*

Species	Family	References
Pyrrhocoris apterus	Pyrrhocoridae	Kodrik *et al.* (2000)
Lamarkiana sparrmani	Pyrgomorphidae	Gaede (2006)
Dysdercus intermedius	Pyrgomorphidae	Kodrik *et al.* (2010)
Iphita limbata	Pyrrhocoridae	Present study

(Gaede *et al.*, 2007) showed that Pyrap-AKH is found in an orthopteran (Family: Pyrgomorphidae) suggests the possibility of the presence of this peptide in other insect groups also (Table 1) thus suggesting the phylogenetic relationship between insect groups. Phylogenetic studies using molecular markers (mitochondrial DNA) have been successfully conducted in horse crab (Kamaruzzaman *et al.*, 2011) and cereal aphids (Helmi and Khafaga, 2011). Similar studies may be useful to obtain more clarity about the heteropteran phylogeny. Recently, Kodrik *et al.* (2002b) identified another AKH/RPCH peptide from the CC of firebug, *P. apterus*, the Peram-CAH-II. In the present study, HPLC separation and further fractionation of extracts of *Iphita* retrocerebral complexes indicated the presence of more than one adipokinetic factors in them. However, by MALDI-MS analysis, we could not find any molecular mass corresponding to Peram-CAH-II in *Iphita*. This might be due to the fact that these peptides are either absent in *I. limbata* or are present in a concentration below the detection limit of the instrument we used.

CONCLUSION

The present investigation demonstrated the presence of neuropeptide hormone, Pyrap-AKH in the retrocerebral extract of the plant bug, *Iphita limbata*. The results also demonstrate a family specificity in the distribution of Pyrap-AKH within Heteroptera.

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REFERENCES

Ajaykumar, A.P. and M. Gokuldas, 2011. Elucidation of primary structure of an adipokinetic neuropeptides from the coffee locust *Aularches miliaris* L. (Orthoptera: Pyrgomorphidae). Int. J. Biol. Chem., 5: 127-135.

Alquicer, G., D. Kodrik, N. Krishnan, J. Vecera and R. Socha, 2009. Activation of insect antioxidant mechanism by mammalian glucagon. Comp. Biochem. Physiol., 152: 226-227.

Brown, A.E. and A.N. Starratt, 1975. Isolation of proctolin, a myotropic peptide, from *Periplaneta americana*. J. Insect Physiol., 21: 1879-1881.

Carlisle, J. and B.G. Loughton, 1979. Adipokinetic hormone inhibits protein synthesis in *Locusta*. Nature, 282: 420-421.

Diez, J.J. and P. Iglesias, 2003. The role of novel adipocyte derived hormone adiponectin in human disease. Eur. J. Endocrinol., 148: 293-300.

Fabold, K., H.I. H. El-Damanhouri and M.W. Lorenz, 2010. Age-dependent cyclic locomotor activity in the cricket, *Gryllus bimaculatus* and the effect of adipokinetic hormone on locomotion and excitability. J. Comp. Physiol. A. Neuroethol. Sens. Neural Behav. Physiol., 196: 271-283.

Frings, C.S., T.W. Fendly, R.T. Dunn and C.A. Queen, 1972. Improved determination of total serum lipids by the sulfo-phospho-vanillin reaction. Clin. Chem., 18: 673-674.

Gaede, G., L. Auerswald, P. Simek, H.G. Marco and D. Kodrik, 2003. Red pigment concentrating family hormone is not limited in crustaceans. Biochem. Biophy. Res. Commun., 309: 967-973.

Gaede, G., 2004. Flight or fight-the need for adipokinetic hormones. Int. Congr. Ser., 1275: 134-140.

Gaede, G., 2006. Unusual compliment of three octapeptides in two species of grasshoppers (Caelifera). Eur. J. Entomol., 103: 297-304.

Gaede, G., P. Simek and H. Marco, 2007. Water scorpions (Heteroptera, Nepidae) and giant water bugs (Heteroptera, Belostomatidae): Sources of new members of the adipokinetic hormone/red pigment concentrating hormone family. Peptides, 28: 1359-1367.

Gaede, G. and H.G. Marco, 2009. Peptides of the adipokinetic hormone/red pigment concentrating family with special emphasis on Caelifera: Primary sequences and functional considerations contrasting grasshoppers and locusts. Gen. Comp. Endocrinol., 162: 59-68.

Gokuldas, M., P.A. Hunt and D.J. Candy, 1988. Inhibition of lipid synthesis *in vitro* in the locust, *Schistocerca gregaria*, by a factor from *Corpora cardiaca*. Physiol. Entomol., 13: 43-48.

Goldsworthy, G.J., 1983. The Endocrine Control of Flight Metabolism in Locusts. In: Advances in Insect Physiology, Berridge, M.J., J.E. Treherne and V.B. Wigglesworth (Eds.). Academic Press, New York, pp: 149-204.

Helmi, A. and A.F. Khafaga, 2011. Molecular fingerprinting of certain cereal aphids in Egypt (Hemiptera: Sternorrhyncha: Aphididae) Using RAPD and ISSRs markers. J. Entomol., 8: 327-340.

Kamaruzzaman, B.Y., B.A. John, K. Zaleha and K.C.A. Jalal, 2011. Molecular phylogeny of horseshoe crab. Asian J. Biotechnol., 3: 302-309.

Kodrik, D. and G.J. Goldsworthy, 1995. Inhibition of RNA synthesis by adipokinetic hormones and brain factor (s) in adult fat body of *Locusta migratoria*. J. Insect Physiol., 41: 127-133.

Kodrik, D., R. Socha, P. Simek, R. Zemek and G.J. Goldsworthy, 2000. A new member of the AKH/RPCH family that stimulates locomotory activity in the firebug. *Pyrrhocoris apterus* (Heteroptera). Insect Biochem. Mol. Biol., 30: 489-498.

Kodrik, D., R. Simek, L. Lepsa and R. Socha, 2002a. Identification of the cockroach neuropeptide Peram-CAH-II as a second adipokinetic hormone in the firebug, *Pyrrhocoris apterus*. Peptides, 23: 585-587.

Kodrik, D., R. Socha and R. Zemek, 2002b. Topical application of Pyrap-AKH stimulates lipid mobilization and locomotion in the flightless bug, *Pyrrhocoris apterus* (L.) (Heteroptera). Physiol. Entomol., 27: 15-20.

Kodrik, D., 2008. Adipokinetic hormone functions that are not associated with insect flight. Physiol. Entomol., 33: 171-180.

Kodrik, D., H.G. Marco, P. Simek, R. Socha, P. Stys and G. Gade, 2010. The adipokinetic hormones of Heteroptera: A comparative study. Physiol. Entomol., 35: 117-127.

Lorenz, M.W., 2001. Synthesis of lipids in the fat body of *Gryllus bimaculatus*: Age dependency and regulation by adipokinetic hormone. Arch. Insect Biochem. Physiol., 47: 198-214.

Marco, H.G., 2004. Unity and diversity in chemical signals of arthropod: The role of insect neuropeptides in crustaceans and insects. Int. Congr. Ser., 1275: 126-133.

Rasheed, K.A. and M. Gokuldas, 2002. Evidences for adipokinetic activity in *Iphita limbata* Stal (Pyrrhocoridae: Heteroptera). Entomon., 27: 125-136.

Shcherbakov, D.E. and Y.A. Popov, 2002. Super Order Cimicidae Laicharting. In: History of Insects, Rasnitsyn, A.P. and D.L.J. Quicke (Eds.). Kluwer Academic Publishers, The Netherlands, pp: 143-157.

Shuranova, Z.P., Y.M. Burmistrov, J.R. Strawn and R.L. Cooper, 2006. Evidence for an autonomic nervous system in decapod crustaceans. Int. J. Zool. Res., 2: 242-283.

Socha, R., D. Kodrik and R. Zemek, 1999. Adipokinetic hormone stimulates insect locomotor activity. Naturwissenschaften, 88: 85-86.

Socha, R., D. Kodrik, R. Zemek and M. Patockova, 2004. The kind of AKH-mobilized energy substrates in insects can be predicted without a knowledge of the hormone structure. Eur. J. Entomol., 101: 29-35.

Stone, J.V., W. Mordue, K.E. Batley and H.R. Morris, 1976. Structure of locust adipokinetic hormone, a neurohormone that regulates lipid utilisation during flight. Nature, 263: 207-211.

Tsao, T.S., H.F. Lodish and J. Fruebis, 2002. ACRP30, a new hormone controlling fat and glucose metabolism. Eur. J. Pharmacol., 440: 213-221.

Velmurugan, S., P. Soundarapandian N.J. Samuel and S. Rajagopal, 2008. Activity of hyperglycemic hormone in the eyestalk of commercially important crab *Portunus pelagicus* (Linnaeus). J. Fish. Aquatic Sci., 3: 77-81.