Peptides 31 (2010) 498–505

Contents lists available at ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Antifeedant activity and high mortality in the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphidae) induced by biostable insect kinin analogs

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ARTICLE INFO

Article history:
Received 27 May 2009
Received in revised form 30 June 2009
Accepted 1 July 2009
Available online 9 July 2009

Keywords:
Kinin analog
Antifeedant
Aphicide
Acyrthosiphon pisum
Insect
Gut
Myotropic
Diuresis
Peptidase resistant

ABSTRACT

The insect kinins are multifunctional neuropeptides found in a variety of arthropod species, including the pea aphid *Acyrthosiphon pisum* (Hemiptera: Aphidae). A series of biostable insect kinin analogs based on the shared C-terminal pentapeptide core region were fed in solutions of artificial diet to the pea aphid over a period of 3 days and evaluated for antifeedant and aphicidal activity. The analogs contained either α,α-disubstituted or β-amino acids in key positions to enhance resistance to tissue-bound peptidases and retain activity in a number of insect kinin bioassays and/or on expressed receptors. Three of the biostable analogs demonstrated antifeedant activity, with a marked reduction in honeydew formation observed after 1 day, and very high mortality. In contrast, an unmodified, parent insect kinin and two other analogs containing some of the same structural components that promote biostability are inactive. The most active analog, double Aib analog **K-Aib-1** ([Aib]FF[Aib]WGa), featured aphicidal activity calculated at an LC_{50} of 0.063 nmol/μl (0.048 μg/μl) and an LT_{50} of 1.68 days, matching the potency of some commercially available aphicides. The mechanism of this activity has yet to be established. The aphicidal activity of the biostable insect kinin analogs may result from different potential mechanisms as disruption of digestive processes by interfering with gut motility patterns, digestive enzyme release, and/or with fluid cycling in the gut, and also nutrient transport across the gut itself; all processes shown to be regulated by the insect kinins in other insects. However the mechanism(s) is(are) not yet known. The active insect kinin analogs represent potential leads in the development of selective, environmentally friendly pest aphid control agents.

1. Introduction

The insect kinins are multifunctional neuropeptides found in several arthropod and invertebrate groups [3,9,34,36,50]. They were first isolated from the cockroach, *Leucophaea maderae*, according to their myostimulatory activity on hindgut contraction [18–20,28]. Shortly after their discovery, insect kinins were shown to have diuretic activity on isolated Malpighian tubules of the yellow fever mosquito *Aedes aegypti* and the cricket *Acheta domesticus* [4,15]. Insect kinins, and/or analogs, have subsequently been reported to modulate digestive enzyme release [13,14,20,33,28,41], and interestingly they can also inhibit weight gain in larvae of the tobacco budworm (*Heliothis virescens*) and corn earworm (*Helicoverpa zea*), both serious pest insects in agriculture [26,33,41].

The insect kinins share the evolutionarily conserved C-terminal pentapeptide motif Phe-X^1-X^2-Trp-Gly-NH_2, where X^1 = His, Asn, Ser or Tyr, and X^2 = Ser, Pro or Ala [17,50]. The C-terminal pentapeptide kinin core is the minimum sequence required for full cockroach myotropic and cricket diuretic activity in assays with tissues [26,28] and for bioluminescence response in CHO-K1 cells expressing kinin receptors [16,35,46]. Activity in these assays and the receptor expressing system is completely lost when the C-terminal amide of the insect kinin is replaced with a negatively charged acid moiety [27,46]. Evaluation of an Ala-replacement analog series of the C-terminal pentapeptide confirmed the importance of Phe^1 and Trp^4 side chains, because the replacement of these two positions with Ala also leads to the complete loss of activity on mosquito and tick receptor expressing systems and in myotropic and diuretic assays. These studies also show that the variable position 2 tolerates a wide range of chemical characteristics, from acid to basic residues and from hydrophilic to hydrophobic, although aromatic residues at this position are associated with the highest potencies in Malpighian tubule fluid secretion assays [28,37,46]. The natural achetakinins elicit cricket Malpighian tubule fluid secretion at EC_{50} values ranging from 18 to

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0196-9781/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.peptides.2009.07.001

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
JUN 2009		**00-00-2009 to 00-00-2009**

4. TITLE AND SUBTITLE
Antifeedant activity and high mortality in the pea aphid Acyrthosiphon pisum (Hemiptera: Aphidae) induced by biostable insect kinin analogs

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Department of Agriculture ,Areawide Pest Management Research,Southern Plains Agricultural Research Center,College Station,TX,77845

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

The insect kinins are multifunctional neuropeptides found in a variety of arthropod species, including the pea aphid Acyrthosiphon pisum (Hemiptera: Aphidae). A series of biostable insect kinin analogs based on the shared C-terminal pentapeptide core region were fed in solutions of artificial diet to the pea aphid over a period of 3 days and evaluated for antifeedant and aphicidal activity. The analogs contained either a,a-disubstituted or b-amino acids in key positions to enhance resistance to tissue-bound peptidases and retain activity in a number of insect kinin bioassays and/or on expressed receptors. Three of the biostable analogs demonstrated antifeedant activity, with a marked reduction in honeydew formation observed after 1 day, and very high mortality. In contrast, an unmodified, parent insect kinin and two other analogs containing some of the same structural components that promote biostability are inactive. The most active analog, double Aib analog K-Aib-1 ([Aib]FF[Aib]WGa), featured aphicidal activity calculated at an LC50 of 0.063 nmol/ml (0.048 mg/ml) and an LT50 of 1.68 days, matching the potency of some commercially available aphicides. The mechanism of this activity has yet to be established. The aphicidal activity of the biostable insect kinin analogs may result from different potential mechanisms as disruption of digestive processes by interfering with gut motility patterns, digestive enzyme release and/or with fluid cycling in the gut, and also nutrient transport across the gut itself; all processes shown to be regulated by the insect kinins in other insects. However the mechanism(s) is(are) not yet known. The active insect kinin analogs represent potential leads in the development of selective environmentally friendly pest aphid control agents.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**8**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

325 pmol/l [4]. The active core pentapeptide is equipotent with the parent non-peptide in this assay. Due to kinin peptide susceptibility to both exo- and endopeptidases in the insect hemolymph and gut, insect kinin peptides cannot be directly used as pest control agents and/or research tools by insect neuroendocrinologists. Members of the insect kinin family are hydrolyzed, and therefore inactivated, by tissue-bound peptidases of insects [5,10,24]. Two susceptible hydrolysis sites in insect kinins have been reported in the active core sequence Phe^1-Tyr^2-Pro^3-Trp^4-Gly^5-NH_2. The primary site is between Pro^3 and the Trp^4 residue, with a secondary site N-terminal to the Phe^1 residue in natural extended insect kinin sequences [33,52]. It has been demonstrated experimentally that the primary hydrolysis site is susceptible to cleavage by angiotensin converting enzyme (ACE) from the housefly and both the primary and secondary hydrolysis sites can be cleaved by neprilysin (NEP) [5,23,24,29,32,33,37].

To overcome the limitations inherent in the physicochemical characteristics of peptides, the development of peptidomimetic analogs has become an important strategy for enhancing their potential as agents for pest control. It has been proposed that blocking or overstimulating the receptors of insect neuropeptides could lead to reduction of pest fitness or death [10,31]. Peptidomimetics is a broader term used to refer to pseudopeptides and non-peptides designed to perform the functions of a peptide. Generally these peptidomimetics are derived by the structural modification of the lead peptide sequence to overcome a number of limitations, such as proteolytic degradation that restrict the use of peptides as insect pest control agents [31,45]. One peptidomimetic approach is the incorporation of sterically hindered α,α-disubstituted amino acids such as α-amino-isobutyric acid (Aib) or an α-methyl-phenylalanine residue (α-MePhe). Several of these analogs have demonstrated agonist responses that match or exceed the potency of native insect kinins in *in vitro* Malpighian tubule fluid secretion assays of the cricket *A. domesticus* and mosquito *A. aegypti*, an *in vivo Musca domestica* housefly diuretic assay, as well as on receptor expressing systems of the tick *Boophilus microplus* and mosquito *A. aegypti* [29,33,46,48]. They have also demonstrated enhanced resistance to the endopeptidases ACE and NEP, enzymes that deactivate the natural insect kinins as well as evidence of longer hemolymph residence times in the housefly. An alternate peptidomimetic approach is the incorporation of a β-amino acid. The β-amino acid is similar to an α-amino acid in that they both contain an amino terminus and a carboxyl terminus. However, in a β-amino acid an additional methylene group (–CH_2–) is placed between the α-carbon and the acid group (designated as β^3) [2]. By incorporating a β-amino acid, many peptides not only retain their biological activity but also demonstrate enhanced resistance to degradation by peptidases [21]. Indeed, β-amino acid analogs of the insect kinins have demonstrated enhanced resistance to the endopeptidases ACE and NEP, enzymes that deactivate the natural insect kinins [52]. As β-amino acids feature more degrees of freedom (*i.e.*, greater flexibility), analogs incorporating them are generally able to adopt the same and/or similar conformations as those observed in the parent α-amino acid peptides [2,21]. Several of these β-amino acid insect kinin analogs matched the potency range of native achetakinins in an *in vitro* Malpighian tubule fluid secretion assay in the cricket *A. domesticus* [52] and positive insect kinin controls in receptor expressing systems of the tick *B. microplus* and mosquito *A. aegypti* [47].

To date about 4000 aphid species have been described, and about 250 are serious pests to various crops and ornamental plants around the world, causing both direct damage to plants and indirect damage by transmitting important viruses that can devastate agricultural crops [1]. Specifically, the pea aphid *Acyrthosiphon pisum* (Hemiptera, Aphidae) alone causes hundreds of millions of dollars of crop damage every year, and many populations have already acquired resistance towards multiple conventional and modern insecticides, making a search for alternative strategies urgent [7]. Furthermore aphids are not sensitive to the toxins from the bacterium *Bacillus thuringiensis* (*Bt*) [43]. Interestingly, the 525Mb genome of *A. pisum* has recently been sequenced by the International Aphid Genomic Consortium providing a resource for comparative genomics and the tools to identify targets for control (AphidBase; http://www.aphidbase.com; [49]). We identified the sequence of the native aphid kinin as ASDKHGRP-Lys-Gln-Thr-*Phe*-Ser-*Ser-Trp-Gly-*NH_2.

In this manuscript, the five biostable insect kinin analogs listed below that incorporate modifications (α,α-disubstituted amino acids, β-amino acids, or both) of the C-terminal pentapeptide core region of the insect kinins were fed in solutions of artificial diet to the pea aphid *A. pisum* over a period of 3 days and evaluated for antifeedant and aphicidal activity.

K-Aib-1: **Aib**-Phe-Phe-**Aib**-Trp-Gly-NH_2
K-Aib-2: α**MePhe**-Phe-Ser-**Aib**-Trp-Gly-NH_2
K-Aib-4: **Ac**-Arg-β^3**Phe**-Phe-Phe-**Aib**-Trp-Gly-NH_2
K-βA-2: **Ac**-Arg-β^3**Phe**-Phe-Phe-β^3**Pro**-Trp-Gly-NH_2
K-βA-1: **Ac**-Arg-Phe-Phe-β^3**Pro**-Trp-Gly-NH_2
[K stands for kinin analog]

All analogs that did not feature a sterically hindered α,α-disubstituted amino acid at the N-terminus were also blocked with an acetyl (Ac) group, which confers resistance to hydrolytic degradation by an additional class of peptidases, the aminopeptidases [12]. An unmodified insect kinin peptide (Phe-Phe-Phe-Ser-Trp-Gly-NH_2) served as a positive control.

2. Materials and methods

2.1. Synthesis and characterization of Aib-containing and β-amino acid kinin analogs

The Aib-containing insect kinin analogs **K-Aib-1** (**Aib**-Phe-Phe-**Aib**-Trp-Gly-NH_2), **K-Aib-2** (α**MePhe**-Phe-Ser-**Aib**-Trp-Gly-NH_2), and **K-Aib-4** (**Ac**-Arg-β^3**Phe**-Phe-Phe-**Aib**-Trp-Gly-NH_2) and the unmodified insect kinin analog Phe-Phe-Phe-Ser-Trp-Gly-NH_2 were synthesized, purified and quantified as previously described by Taneja-Bageshwar et al. [48]. The β-amino acid insect kinin analogs **K-βA-2** (**Ac**-Arg-β^3**Phe**-Phe-Phe-β^3**Pro**-Trp-Gly-NH_2) and **K-βA-1** (**Ac**-Arg-Phe-Phe-β^3**Pro**-Trp-Gly-NH_2) were synthesized, purified and quantified as previously described by Zubrzak et al. [52]. Both sets of analogs were synthesized and purified at the Areawide Pest Management Research Unit, Southern Plains Agricultural Research Center, USDA, College Station, TX, USA.

2.2. Insect rearing

A continuous colony with all stages of the pea aphid *A. pisum* was maintained on young broad bean (*Vicia faba*) plants in the Laboratory of Agrozoology at Ghent University, Belgium, under standardized conditions of 25 ± 2 °C, a 16 h light photoperiod and 65 ± 5% relative humidity. Mature aphids were put on plants for 24 h, resulting in a synchronized offspring, *i.e.*, neonate nymphs with an age of 0–24 h that were used throughout the experiments [38].

2.3. Bioassay with pea aphid in a feeding apparatus with an artificial assay to determine antifeedant and aphicide activity by kinin analogs

As food for the aphids, a standard diet previously developed for *A. pisum* [8] was used as the basal diet to which the peptide

analogs were added. The feeding apparatus was prepared using plexiglass cylinders (3 cm high and 3 cm diameter). The food sachet was made under sterile conditions and consists of two layers of parafilm membrane on top of the container. About 200 μl of the artificial diet was sandwiched between the two layers [38].

To challenge aphids to the insect kinin peptide analogs, a stock solution was prepared in the solvent 80% acetone/0.01% TFA, and then diluted at a maximum of 20 μl stock solution in 200 μl the artificial diet to prepare different concentrations between 0.001 and 0.500 nmol/μl. The pH of the artificial diet was neutral in all controls and treatments with a pH 7.5 ± 0.1. In the treatments, 200 μl of each concentration was used to make a food sachet. In the solvent-controls the diet was supplemented with an amount of the solvent 80% acetone/0.01% TFA as for the treatment group, and in the blank-controls with distilled water. At day 0, 20 neonate nymphs of 0–24 h in age (as described previously) were transferred onto the artificial diet. For each concentration, three replicates were carried out and aphids were checked daily during 3 days for honeydew formation to determine antifeedant effects and also for numbers of dead aphids to determine aphicidal effects. The experiment was performed two times independently from each other.

To determine antifeedant effects, the amounts of honeydew produced by the aphids in the treatments as compared to controls were measured using the Ninhydrin test as described by Kanrar et al. [22]. In brief, a 3.6 cm-diameter petri dish, as described above in the feeding apparatus, was lined with a Whatman No. 3 filter paper. This filter paper (with the honeydew) was removed at 24 h after feeding and sprayed with 0.2% ninhydrin reagent to detect the presence of honeydew spots.

The aphid mortality percentages were analyzed using non-linear sigmoid curve fitting, and the toxicity of each treatment was evaluated on the basis of time–response curves and concentrations–response using the GraphPad Prism 4.0 software (GraphPad Software Inc., San Diego, CA). We estimated the median LT_{50} and LC_{50} values with their corresponding 95% confidence interval, which is the time period of feeding on treated diet needed to kill 50% of the aphids and the concentration of the kinin analog needed to kill 50% of the aphids, respectively [44]. The mortality data were corrected according to Abbott's formula based on the mortality seen in the control groups; in all experiments, mortality in the control groups averaged at a low level of 7%.

3. Results

3.1. Effect of Aib-containing insect kinin analogs on pea aphids

Different concentrations of the kinin analogs **K-Aib-1–4** were added to artificial diet and tested for antifeedant and aphicide activity. It was clear that the antifeedant effect of the kinin analogs was rapid. As shown in Fig. 1, the amounts of honeydew formation were markedly reduced already in the first day of treatment with the disubstituted kinin analog **K-Aib-1** at 0.5 nmol/μl. At day 2, The LT_{50} or the time of feeding on the treated diet treated needed to kill 50% of the aphids was estimated to be 1.68 days (Fig. 2A). This trend was also visible for **K-Aib-2**, but to a lower extent. In these conditions we observed normal piercing and probing behavior of the aphids on the food sachets with treated diet, suggesting that ingestion is taking place. Further on, there was no recovery of the feeding during the experiment, and all the intoxicated aphids died. In contrast, honeydew formation with the unmodified insect kinin (tested up to 0.2 nmol/μl) and **K-Aib-4** (tested up to 0.1 nmol/μl) were similar to the controls (data not shown).

From the series tested, it was clear that the disubstituted Aib-1-containing kinin analog **K-Aib-1** was the most toxic for the pea aphids as at 0.5 nmol/μl all aphids were dead (90–100%). With the use of sigmoid curve analysis, an LC_{50} value of 0.063 nmol/μl was calculated for **K-Aib-1** (Fig. 2B, Table 1). In great contrast, the unmodified insect kinin was inactive as there was 100% survival of the aphids with the highest concentrations tested (0.20 nmol/μl). For the other α,α-disubstituted kinin analog, **K-Aib-2** consisting of an αMePhe and one Aib, was also active but lower than **K-Aib-1**; its LC_{50} was calculated 0.086 nmol/μl (Table 1) with Prism software. The hybrid analog **K-Aib-4** with a β^3Phe and one Aib, was considered as inactive as there was only 22% mortality with the highest concentration of 0.1 nmol/μl. With sigmoid curve analysis an LC_{50} value of 0.063 nmol/μl was calculated for **K-Aib-1** (Fig. 2B, Table 1).

3.2. Effect of β-amino acid insect kinin analogs against pea aphids

For the double β^3-protected kinin analog **K-βA-2** we scored antifeedant and aphicide activities. When pea aphids were fed with 0.1 nmol/μl (data not shown) of the double β^3-protected kinin analog **K-βA-2**, the amount of honeydew was reduced between day 1 and day 2 in a similar manner as for **K-Aib-1** and **K-Aib-2** (above). When feeding for 1, 2 and 3 days on diet with 0.1 nmol/μl,

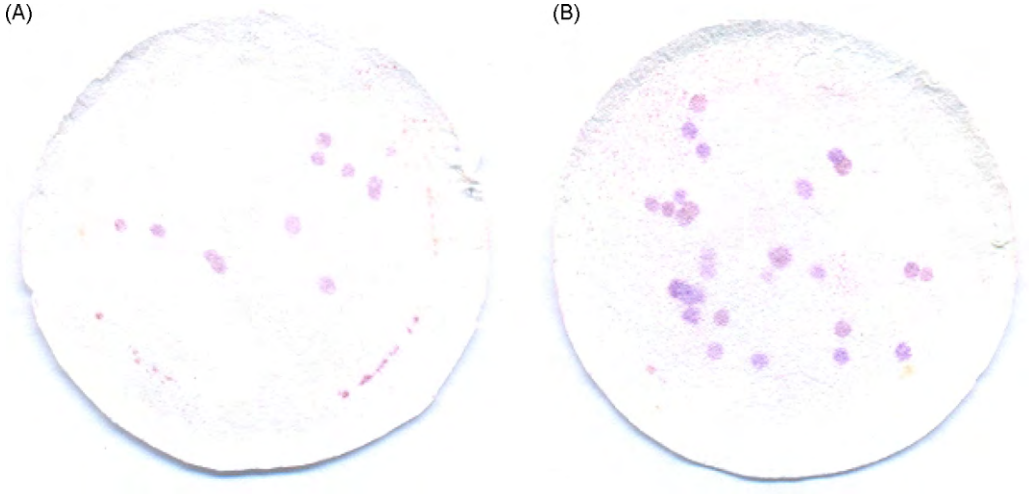

Fig. 1. Treatment of pea aphid *Acyrthosiphon pisum* during 1 day with the double Aib-containing insect kinin peptide analog **K-Aib-1** at 0.5 nmol/μl (A) in the artificial diet caused a remarked reduction in the amounts of honeydew produced by aphid nymphs, as compared to the control (B) after visualization by use of the Ninhydrin test.

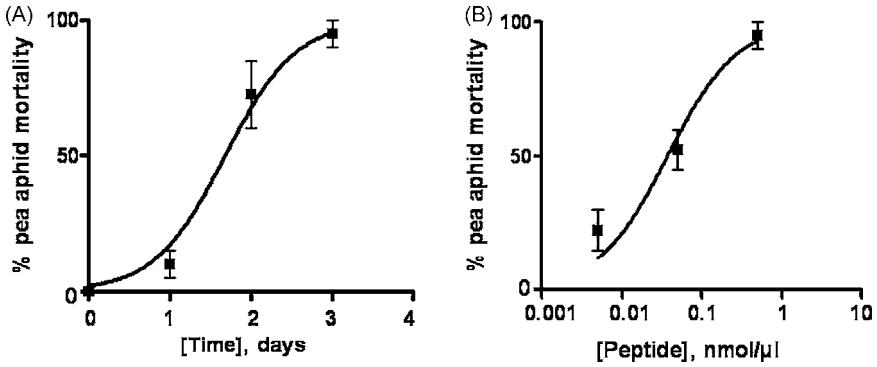

Fig. 2. Induction of aphid mortality by the double Aib-containing insect kinin peptide analog **K-Aib-1** in the pea aphid *Acyrthosiphon pisum*. (A) Time–response over the three days of feeding of aphids on treated diet with 0.5 nmol/µl of the **K-Aib-1**, and (B) concentration–response curve for mortality of aphids by different concentrations of **K-Aib-1** when fed for 3 days via treated diet. The unmodified insect kinin FFFSWGa was inactive. Mortality percentages are based on two repeated experiments, each consisting of 3 groups of 20 nymphs each; a total of 120 aphids were tested per concentration. Statistical analysis and graphs were generated with the GraphPad Prism 4.0 software.

Table 1
Aphid mortality LC_{50} values (expressed as nmol/µl and µg/µl in the artificial diet) for the five insect kinin analogs and the unmodified kinin against the pea aphid *Acyrthosiphon pisum*.

Insect kinin analogs		Aphid mortality (LC_{50} in diet)	
		nmol/µl	µg/µl
Aphid kinin	ASDKHGRP-Lys-Gln-Thr-*Phe*-Ser-*Ser-Trp-Gly-*NH_2	–	
Unmodified insect kinin	Phe-*Phe*-Phe-*Ser-Trp-Gly-*NH_2	Inactive	
K-Aib-1	**Aib**-*Phe*-Phe-**Aib**-*Trp-Gly-*NH_2	0.063	0.046
K-Aib-2	α**MePhe**-*Phe*-Ser-**Aib**-*Trp-Gly-*NH_2	0.085	0.068
K-Aib-4	**Ac**-Arg-β^3**Phe**-*Phe*-Phe-Aib-*Trp-Gly-*NH_2	Inactive	
K-βA-2	**Ac**-Arg-β^3**Phe**-*Phe*-Phe-β^3**Pro**-*Trp-Gly-*NH_2	0.119	0.122
K-βA-1	**Ac**-Arg-*Phe*-Phe-β^3**Pro**-*Trp-Gly-*NH_2	Inactive	

mortality was 5 ± 4%, 32 ± 5% and 70 ± 11% mortality, respectively. At day 3, Prism software calculated an LC_{50} of 0.122 nmol/µl for **K-βA-2** (Table 1). In great contrast, the single β^3-protected kinin analog **K-βA-1** was not active as there was 100% survival of the aphids after 3 days with the highest concentrations tested (0.10 nmol/µl), as was also the case in the controls.

4. Discussion

In the current study, the most impressive result is that a double Aib-containing kinin peptide (**K-Aib-1**) elicited strong activity against whole aphid insects causing mortality, whereas the unmodified insect kinin analog (FFFSWGa) was inactive. In previous work, the replacement of Ser or Pro within the C-terminal insect kinin C-terminal pentapeptide core with a sterically hindered Aib residue has been proposed to enhance resistance to cleavage by tissue-bound peptidases (including ACE) between Ser^3 (or Pro^3) and the Trp^4 residue [29]. The core analog Phe-Phe-**Aib**-Trp-Gly-NH_2 demonstrates complete resistance to degradation by *Musca* ACE over the 120 min experimental period. Molecular dynamics calculations indicate that this Aib-containing core analog mimics a critical β-turn conformation over the C-terminal pentapeptide core residues 1–4 that represents the receptor interaction model for the insect kinins (Fig. 3A and B) [25,30,37]. The core Aib analog demonstrates fluid secretion stimulatory activity in an *in vitro* cricket Malpighian tubule assay that exceeds the range of potency for the native achetakinins, whereas it falls in the middle of the potency range of these same

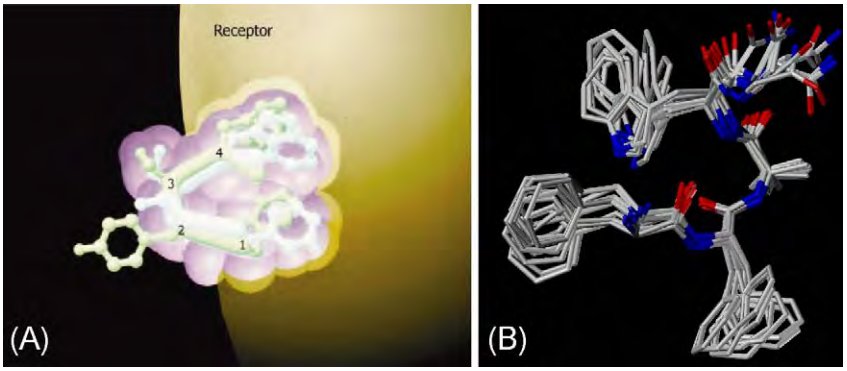

Fig. 3. (A) The currently accepted receptor interaction model of an insect kinin C-terminal pentapeptide core region in a 1–4 β-turn, the active conformation. The side chains of the critical residues Phe^1 and Trp^4 interact to form an aromatic surface that interacts with the receptor site [28,30,32,33,37] (Fig. 3A from Roberts et al. [37]). (B) Molecular dynamics calculations on the insect kinin C-terminal pentapeptide core analog Phe-Phe-Aib-Trp-Gly-NH_2, incorporating a sterically hindered Aib residue in place of Ser^3 (or Pro^3), indicate that the lowest energy conformation mimics the 1–4 β-turn of the receptor interaction model depicted in Fig. 3A (Fig. 3B from Moyna et al. [25]).

natural insect kinins for myostimulatory activity in the *in vitro* cockroach hindgut contractile assay [29,33]. In an *in vitro* digestive enzyme release bioassay in the lepidopteran coconut pest *Opisina arenosella*, this core Aib analog mimicked the amylase (carbohydrase) release inhibition activity of natural insect kinins, specifically demonstrating 1.1- and 0.45-fold the activity of leucokinin-II (DPGFSSWGa) and leucokinin-III (DQGFNSWGa), respectively, at a 1 nmol/ml concentration [14]. Studies in cell lines expressing the tick and mosquito receptors indicate that the core Aib insect kinin analog retains significant potency, demonstrating more potency than the control agonist Phe-Phe-Phe-Ser-Trp-Gly-NH_2 (FFFSWGa) in tick receptor cell lines and approaching the potency of the same control agonist in mosquito receptor cell lines [46]. However, this analog is significantly less potent than the native *Aedes* kinins, as the mosquito receptor prefers analogs that are extended somewhat beyond the C-terminal pentapeptide core.

Furthermore, while this core Aib analog protects the primary hydrolysis susceptible site, it cannot protect the secondary site in extended analogs. The N-terminus of this short analog is vulnerable as well to hydrolysis by aminopeptidases, which also leads to inactivation. For these reasons, other Aib-containing analogs that incorporate a second modification (either a second α,α-disubstituted amino acid or a β-amino acid) to the residue N-terminal to Phe[1] of the core have been prepared to protect the secondary hydrolysis susceptible site [33,48]. For instance, although a non-protected insect kinin was degraded within 1 h by tissue-bound peptidases, the disubstituted Aib kinin analog [Aib]FS[Aib]WGa, closely related to the sequence of **K-Aib-1** used in this study, was found to be completely impervious even up to 4 h, at which time the experiment was terminated [33]. In addition, each of the modified analogs used in this study feature a sequence that either terminates in a bulky α,α-disubstituted amino acid (such as Aib or αMePhe) or is capped at the N-terminus with an acetyl group to prevent hydrolysis by aminopeptidases. The analogs **K-Aib-1**, **K-Aib-2** and **K-Aib-4** used in this study demonstrate a highly significant increase in resistance to hydrolysis by peptidases ANCE, neprilysin, and Leucine aminopeptidase (aminopeptidase M) as compared with the standard kinin FFFSWGa [48]. The analog **K-Aib-4**, capped with an acetyl group on the N-terminus, was 95-fold less susceptible to hydrolysis by aminopeptidase. Analogs **K-Aib-1** and **K-Aib-2** that feature sterically hindered α,α-disubstituted residues at the N-terminus also demonstrate resistance to hydrolysis by the aminopeptidase, with rates of hydrolysis being 32- and 244-fold lower, respectively, than the standard insect kinin sequence [48]. Challenged with NEP, analogs **K-Aib-1**, **K-Aib-2** and **K-Aib-4** were 17-, 5- and 10-fold less susceptible to hydrolysis than the standard kinin FFFSWGa, respectively. Analogs **K-Aib-1**, **K-Aib-2** and **K-Aib-4** were 24-, 9- and 45-fold less susceptible to hydrolysis by ANCE than the standard kinin FFFSWGa, respectively [48].

Two other biostable analogs used in this study feature β-amino acids adjacent to either one or both of the primary and/or secondary peptidase susceptible sites in the insect kinin core. Analogs **K-βA-1** and **K-βA-2** are capped by an acetyl group to enhance resistance to aminopeptidases and only 15% and 0%, respectively, are hydrolyzed by NEP in the period of time that is required to degrade 100% of the natural insect kinin LK-I [52]. During a period of time in which 82% of LK-I is degraded, only 11% and 4%, respectively, of analogs **K-βA-1** and **K-βA-2** are hydrolyzed [52].

Although data on the activity of natural and/or modified insect kinin analogs have not been previously collected on *in vitro* bioassays of the pea aphid, data on the evaluations of the standard insect kinin and biostable analogs used in this study have been obtained for diuretic and/or expressed receptor bioassays in several other arthropod species. On the *B. microplus* tick (BmLK3) receptor transfected cell line assay, analogs **K-Aib-1**, **K-Aib-2**, **K-Aib-4**, **K-βA-1** and **K-βA-2** were 5.5-, 2.5-, 1.3-, 0.95- and 0.55-fold the potency of the control kinin FFFSWGa, respectively [48] (Table 2). On the *A. aegypti* mosquito (E10) receptor transfected cell line assay analogs **K-Aib-1**, **K-Aib-2**, **K-Aib-4**, **K-βA-1** and **K-βA-2** were 8-, 2-, 1.5-, 1.7-, and 0.95-fold the potency of the control kinin FFFSWGa, respectively; and 2-, 0.6-, 0.4-, 0.45- and 0.25-fold the potency of the native *Aedes* kinin-2 (NPFHAWGa) (Table 2), respectively. In addition, analog **K-Aib-1** matches the activity of *Aedes* kinin-1 (NSKYVSKQKFYSWGa) (that is more potent than *Aedes* kinin-2) at 0.1 nmol/l in an *in vitro A. aegypti* Malpighian tubule fluid secretion assay [48]. In the *in vitro* cricket Malpighian tubule fluid secretion assay, analogs **K-βA-1** and **K-βA-2** fell within the 20–325 pM (EC_{50}) potency range of the native achetakinins-I–V, whereas **K-Aib-2** (EC_{50} = 0.2 pM) proved to be 100-fold more potent than the most potent achetakinin (Table 2). The analog **Aib**-Phe-Ser-**Aib**-Trp-Gly-NH_2, that differs from the sequence of **K-Aib-1** by only the replacement of a Phe with a Ser, demonstrated an EC_{50} (1 pmol/l) that was 20-fold the potency of the most potent achetakinin [29,33]. This same Aib analog matched the inulin clearance activity of the native muscakinin in an *in vivo* diuretic assay in the housefly [33], even though the *in vitro* diuretic activity in a Malpighian tubule fluid secretion assay was four orders of magnitude less than muscakinin. This

Table 2
Comparative potencies of insect kinin analogs containing α,α-disubstituted and/or β-amino acids, an unmodified control peptide, and insect kinins native to the mosquito *Aedes aegypti* and the cricket *Acheta domesticus* tested on tick (BmLK3) and mosquito (E10) receptor transfected cell lines, as well as an *in vitro* Malpighian tubule fluid secretion assay. It should be noted that the NOVOstar bioluminescence method used to evaluate the response of the insect kinin analogs to expressed receptors in this study is between 50- and 70-fold less sensitive as compared with a less practical fluorescence method that we have previously employed [35]. This difference should be taken into account when estimating the potency that these analogs would likely demonstrate in *in vitro* or *in vivo* physiological bioassays.

Insect kinin analogs	Tick receptor (BmLK3 cell line) EC_{50} ± CI (nmol/l)	Mosquito receptor (E10 cell line) EC_{50} ± CI (nmol/l)	Cricket Malpighian tubule EC_{50} (95%CI) (pmol/l)
K-Aib-1 **Aib**-Phe-Phe-**Aib**-Trp-Gly-NH_2	49 ± 12 [48]	76 ± 18 [48]	
Mosquito *Aedes* kinin-2		164 ± 30 [48]	
Native cricket *Acheta* kinins-I–V			20–325 [33,52]
K-Aib-2 **αMePhe**-Phe-Ser-**Aib**-Trp-Gly-NH_2	111 ± 30 [48]	289 ± 50 [48]	0.2 (0.2–1.7) [33]
K-Aib-4 **Ac**-Arg-β**[3]Phe**-Phe-Phe-**Aib**-Trp-Gly-NH_2	211 ± 30 [48]	411 ± 70 [48]	
K-βA-2 **Ac**-Arg-β**[3]Phe**-Phe-Phe-β**[3]Pro**-Trp-Gly-NH_2	495 ± 50 [47]	653 ± 90 [47]	100 (40–280) [52]
K-βA-1 **Ac**-Arg-Phe-Phe-β**[3]Pro**-Trp-Gly-NH_2	287 ± 40 [47]	367 ± 60 [47]	30 (10–250) [52]
Control kinin Phe-Phe-Phe-Ser-Trp-Gly-NH_2	271 ± 60 [47]	617 ± 50 [47]	

remarkable difference between the *in vitro* and *in vivo* potency of this biostable analog has been attributed to its ability to remain in the hemolymph for extended periods of time [33].

The two reference aphicides that are currently used in the marketplace for selective IPM control against aphids in agriculture are pymetrozine and flonicamid. Both compounds act specifically against aphids as feeding inhibitors, although their exact mechanism(s) remain unidentified. Flonicamid [N-(cyanomethyl)-4-(trifluoromethyl)-3-pyridinecarboxamide] is a novel insecticide; its LC_{50} as determined in an experimental setup similar to that used for the kinin analogs was 0.144 nmol/μl with a typical loss of honey dew formation followed by death, and its LT_{50} was 1.1 days to kill 50% of aphids feeding on diet containing 0.44 nmol/μl (Mahdian and Smagghe, unpublished results). For pymetrozine [1,2,4-triazin-3(2H)-one,4,5-dihydro-6-methyl-4-[(3-pyridinylmethylene)amino], Sadeghi and coworkers calculated with use of a similar feeding apparatus with a diet sachet an LC_{50} of 0.01 μg/ml [39]. The latter authors also tested imidacloprid and found that 50% of aphids were killed (LC_{50}) with 0.03 μg/ml after 3 days of feeding. Imidacloprid is a very active broad-spectrum neonicotinoid insecticide with the nicotinic acetylcholine receptor (nAChR) as target, and to date it is used against a large variety of pest insects and due to its high systemic activity, it is also highly active against sucking pest insects like aphids and whiteflies. But intensive use of imidacloprid has stimulated outbreaks of resistance and cross resistance in many cases [7]. In the group of insect growth regulator (IGRs), azadirachtin (Neem), flufenoxuron and pyriproxyfen are also commercially used in the selective control of aphids and they have a respective LC_{50} of 7.9, 8.7 and 9.3 μg per ml diet against pea aphids [39]. Here typical phenotypic symptoms of aphid mortality were disruption of nymphal molt and abortion of molting. In the field of insecticidal proteins, mannose-binding lectins have received a lot of attention in the last decade, because the *Galanthus nivalis* agglutinin (GNA, homotetrameric protein composed of 12 kDa subunits) is highly detrimental to aphids [6,40,51] and can be delivered via transgenic plants. Sadeghi et al. [38] reported an LC_{50} of 350 and 700 μg/ml for two mannose-binding lectins GNA and ASA after feeding for 3 days on treated diet. In addition, it should be mentioned that aphids are not sensitive to the insecticidal toxins of *Bt* [43]. Thus, the fact that the stabilized insect kinin analogs of this study, and especially the disubstituted **K-Aib-1**, show rapid and high activities against *A. pisum* aphids in the same order of magnitude as some commercial aphicides tested under comparable conditions in the laboratory, suggests that they represent potentially valuable leads for alternative agents in the control of aphids and in the struggle against insecticide resistance. In addition, we believe that testing other sucking pest insects would also be of interest. But before making firm conclusions on their potential value as practical antifeedants, we believe more testing on a larger scale and under more field-related conditions is required.

The aphicidal activity of the insect kinin analogs is associated with the presence of components that enhance the resistance of the C-terminal core region to peptidases, as the unmodified insect kinin demonstrates no activity. In addition, the inactivity of **K-βA-1**, which features only protection of the primary hydrolysis susceptible site, suggests that activity requires protection of the secondary susceptible site and the N-terminal region as well. The presence of structural components that protect both susceptible regions is not in itself sufficient for activity, as analog **K-Aib-4** demonstrates little or no aphicidal effects even though its sterically hindered Aib residue is also present at the same position of the active analogs **K-Aib-1** and **K-Aib-2**, and its β^3Pro residue is similarly present in the same position of active analog **K-βA-1**.

The mechanism of the aphid antifeedant activity and high induction of mortality demonstrated by the biostable insect kinin analogs cannot be clearly identified at this point, but it may be associated with disruption of the physiological processes that this important neuropeptide family regulates in insects. For this to happen, the biostable analogs with aphicidal activity would necessarily need to interact with a native aphid kinin receptor(s). In this respect, it is interesting to note that the absolute order of activity of the three active biostable insect kinin analogs in the aphid assay correlates with that of the potency of interaction with expressed tick and mosquito IK receptors, although the ratios differ. Biostable analog **K-Aib-1** is 2- and 10-fold more potent than **K-Aib-2** and **K-βA-2** in the expressed tick IK receptor, respectively (Table 2). On the expressed mosquito IK receptor, analog **K-Aib-1** is 4- and 9-fold more potent than **K-Aib-2** and **K-βA-2**, respectively (Table 2). Finally, in the aphicidal assay, biostable analog **K-Aib-1** is 1.4- and 2-fold more potent than **K-Aib-2** and **K-βA-2**, respectively (Table 1). Insect kinins and analogs have been shown to stimulate contractions of the hindgut [17,18,28,33], stimulate *in vitro* Malpighian tubule fluid secretion and *in vivo* diuresis [3,4,28,29,33], inhibit the *in vitro* release of the digestive enzyme amylase in the midgut [13,14], and inhibit *in vivo* larval weight gain [26,33,41] in a variety of insects. The aphicidal activity of the biostable insect kinin analogs may therefore result from a disruption of the digestive process by interfering with normal gut motility patterns, digestive enzyme release, and/or interference with normal fluid cycling in the gut. The osmotic pressure of plant phloem sap is generally higher than that of insect body fluids [42]. Pea aphids feature no Malpighian tubules. Nonetheless, water cycling along the length of the gut lumen is believed to contribute to the osmoregulation of aphids and mediated by a membrane-associated aquaporin. The pea aphid aquaporin gene *ApAqp1* expressed in the gut is the ortholog of the *drip* gene (CG9023) that is expressed in the Malpighian tubules of *Drosophila* [42]. Whether the insect kinin analogs may interfere with this water cycling process in the aphid clearly remains to be established. As to larval weight gain inhibition induced by the insect kinins, it has been suggested that this effect may be due to both an increase in diuresis and also an induction of a 'starvation signal' that caused the animals to mobilize their own energy stores and to fail to utilize the digested diet. Experiments by Goldsworthy and coworkers have shown that injected doses of insect kinins can elicit increases in lipid concentrations and reductions in protein levels in the hemolymph of crickets and locusts [11,33].

Finally, it is possible that activation of taste receptors by the presence of the analogs may cause the aphids to avoid ingestion of the diet altogether, leading to starvation. While honeydew formation is depressed in the aphids exposed to the active analogs, the observations of normal piercing behavior and the presence of at least some honeydew suggest that ingestion is nonetheless taking place. Impairment of normal physiological patterns in the aphids ingesting the active analogs may lead to a reduction in subsequent feeding and, in turn, to the observed reduction in levels of honeydew formation. Furthermore, the unmodified peptide is readily ingested by the aphids, as are the essentially inactive analogs **K-βA-1** and **K-Aib-4** which also contain the same unnatural α,α-disubstituted and β-amino acids found in the active analogs. Thus, the fact that these three insect kinin analogs do not trigger avoidance of diet ingestion would seem to suggest that some ingestion of the three active biostable analogs may also be taking place.

As nothing is presently known about the susceptibility of unmodified or biostable insect kinin analogs to digestive enzymes in the aphid gut, future investigations into this question are warranted. While the presence of either the sterically hindered α,α-disubstituted or the structural modifications inherent in β-amino acids likely enhance resistance to digestive peptidases at least to some degree, a better knowledge of the degradation of insect kinin analogs by gut enzymes would facilitate the design of a second generation of antifeedant analogs.

In summary, the presence of three biostable insect kinin core analogs **K-Aib-2**, **K-βA-1** and particularly **K-Aib-1** in the diet demonstrate significant antifeedant activity and induction of high mortality in the pea aphid *A. pisum* that matches that of some commercially available aphicides. An unmodified, parent insect kinin and two other analogs containing some of the same structural components that promote biostability are inactive. The active biostable insect kinin analogs described in this study and/or 2nd generation analogs, either in isolation or in combination with biostable analogs of other neuropeptide classes that also regulate aspects of diuretic, antidiuretic, digestive, reproductive and/or developmental processes, represent potential leads in the development of selective, environmentally friendly pest aphid control agents capable of disrupting those critical processes.

Acknowledgements

The authors wish to thank Allison Strey and Nan Pryor (USDA, College Station) for able technical assistance. We also acknowledge financial assistance from the North Atlantic Treaty Organization (NATO) (RJN) Collaborative Research Grant (#LST.CLG.979226), a grant from the USDA/DOD DWFP Initiative (#0500-32000-001-01R) (RJN), and support from the Fund of Scientific Research (FWO-Vlaanderen, Belgium) to GS.

References

[1] Alford DV. Pest and disease management handbook. Oxford, UK: British Crop Protection Enterprises, Blackwell Science Ltd; 2000.
[2] Cheng RP, Gellman SH, DeGrado WF. Beta-peptides: from structure to function. Chem Rev 2001;101(10):3219–32.
[3] Coast GM, Orchard I, Phillips JE, Schooley DA. Insect diuretic and antidiuretic hormones. Adv Insect Physiol 2002;29:279–341.
[4] Coast GM, Holman GM, Nachman RJ. The diuretic activity of a series of cephalomyotropic neuropeptides, the achetakinins, on isolated Malpighian tubules of the house cricket, *Acheta domesticus*. J Insect Physiol 1990;36:481–8.
[5] Cornell MJ, Williams TA, Lamango NS, Coates D, Corvol P, Soubier F, Hoheisel J, Lehrach H, Isaac RE. Cloning and expression of an evolutionary conserved single-domain angiotensin converting enzyme from *Drosophila melanogaster*. J Biol Chem 1995;270:13613–9.
[6] Down RE, Gatehouse AMR, Hamilton WDO, Gatehouse JA. Snowdrop lectin inhibits development and fecundity of the glasshouse potato aphid (*Aulacorthum solani*) when administered in vitro and via transgenic plants, both in laboratory and glasshouse trials. J Insect Physiol 1996;42:1035–45.
[7] Elbert A, Haas M, Springer B, Thielert W, Nauen R. Applied aspects of neonicotinoid uses in crop protection. Pest Manag Sci 2008;64:1099–105.
[8] Febvay G, Delobel B, Rahbé Y. Influence of amino acid balance on the improvement of an artificial diet for a biotype of Acyrthosiphon pisum (Homoptera: Aphididae). Can J Zool 1988;66:2449–53.
[9] Gade G. Regulation of intermediary metabolism and water balance of insects by neuropeptides. Annu Rev Entomol 2004;49:93–113.
[10] Gade G, Goldsworthy GJ. Insect peptide hormones: a selective review of their physiology and potential application for pest control. Pest Manag Sci 2003;59:1063–75.
[11] Goldsworthy GJ, Coast GM, Wheeler O, Cusinato I, Kay I, Khambay B. The structural and functional activity of neuropeptides. In: Crampton JM, Eggelton P, editors. Royal entomological society symposium on insect molecular science. London: Academic Press; 1992. p. 205–25.
[12] Gregory H, Hardy PM, Jones PM, Kenner DS, Sheppard RC. Antral hormone gastrin. Structure of gastrin. Nature 1964;204:931–3.
[13] Harshini S, Manchu, Sunitha VB, Sreekumar S, Nachman RJ. *In vitro* release of amylase by culekinins in two insects: *Opsinia arenosella* (Lepidoptera) and *Rhynchophorus ferrugineus* (Coleoptera). Trends Life Sci 2003;17:61–4.
[14] Harshini S, Nachman RJ, Sreekumar S. Inhibition of digestive enzyme release by neuropeptides in larvae of *Opisinia arenosella* (Lepidoptera: Cryptophasidae). Comp Biochem Physiol 2002;B132:353–8.
[15] Hayes TK, Pannabecker TL, Hinckley DJ, Holman GM, Nachman RJ, Petzel DH, Beyenbach KW. Leucokinins, a new family of ion transport stimulators and inhibitors in insect malpighian tubules. Life Sci 1989;44:1259–66.
[16] Holmes SP, Barhoumi R, Nachman RJ, Pietrantonio PV. Functional analysis of a G protein-coupled receptor from the southern cattle tick *Boophilus microplus* (Acari:Ixodidae) identifies it as the first arthropod myokinin receptor. Insect Mol Biol 2003;12:27–38.
[17] Holman GM, Nachman RJ, Coast GM. Isolation, characterization and biological activity of a diuretic myokinin neuropeptide from the housefly, *Musca domestica*. Peptides 1999;20:1–10.
[18] Holman GM, Nachman RJ, Wright MS. Comparative aspects of insect myotropic peptides. In: Epple A, Scanes CG, Stetson MH, editors. Progress in comparative endocrinology. New York: Wiley-Liss; 1990. p. 35–9.
[19] Holman GM, Nachman RJ, Wright MS. Insect neuropeptides. Annu Rev Entomol 1990;35:201–17.
[20] Holman GM, Cook BJ, Nachman RJ. Primary structure and synthesis of a blocked myotropic neuropeptide isolated from the cockroach, *Leucophaea maderae*. Comp Biochem Physiol 1986;85:219–24.
[21] Juaristi E, Soloshonok VA, editors. Enantioselective synthesis of β-amino acids. 2nd edition, New York: Wiley; 2005.
[22] Kanrar S, Venkateswari J, Kirti PB, Chopra VL. Transgenic Indian mustard (*Brassica juncea*) with resistance to the mustard aphid (*Lipaphis erysimi* Kalt.). Plant Cell Rep 2002;20:976–81.
[23] Lamango NS, Nachman RJ, Hayes TK, Strey A, Isaac RE. Hydrolysis of insect neuropeptides by an angiotensin converting enzyme from the housefly, *Musca domestica*. Peptides 1997;18:47–52.
[24] Lamango NS, Sajid M, Isaac RE. The endopeptidase activity and the activation by Cl^- of angiotensin-converting enzyme is evolutionarily conserved: purification and properties of an angiotensin-converting enzyme from the housefly, *Musca domestica*. Biochem J 1996;314:639–46.
[25] Moyna G, Williams HJ, Nachman RJ, Scott AI. NMR conformation and solution dynamics of a conformationally constrained insect kinin linear peptide. Biopolymers 1999;49:403–13.
[26] Nachman RJ, Coast GM, Douat C, Fehrentz J, Kaczmarek K, Zabrocki J, Pryor NW, Martinez J, A. C-terminal aldehyde insect kinin analog enhances inhibition of weight gain and induces significant mortality in *Helicoverpa zea* larvae. Peptides 2003;24:1615–21.
[27] Nachman RJ, Coast GM, Holman GM, Beier RC. Diuretic activity of C-terminal group analogues of the insect kinins in *Acheta domesticus*. Peptides 1995;16:809–13.
[28] Nachman RJ, Holman GM. Myotropic insect neuropeptide families from the cockroach *Leucophaea maderae*: structure–activity relationships. In: Menn JJ, Masler EP, editors. Insect neuropeptides: chemistry, biology, and action. Washington, DC: American Chemical Society; 1991. p. 194–214.
[29] Nachman RJ, Isaac RE, Coast GM, Holman GM. Aib-containing analogues of the insect kinin neuropeptide family demonstrate resistance to an insect angiotensin-converting enzyme and potent diuretic activity. Peptides 1997;18:53–7.
[30] Nachman RJ, Kaczmarek K, Williams HJ, Coast GM, Zabrocki J. An active insect kinin analog with 4-aminopyroglutamate, a novel cis-peptide bond, type VI β-turn motif. Biopolymers 2004;75:412–9.
[31] Nachman RJ, Roberts VA, Holman GM, Haddon WF. Leads for insect neuropeptide mimetic development. Arch Insect Biochem Physiol 1993;22:181–97.
[32] Nachman RJ, Roberts VA, Holman GM, Tainer JA. Concensus chemistry and conformation of an insect neuropeptide family analogous to the tachykinins. In: Epple A, Scanes CG, Stetson MH, editors. Progress in comparative endocrinology, Vol. 342. New York: Wiley-Liss, Inc.; 1990. p. 60–6.
[33] Nachman RJ, Strey A, Isaac E, Pryor N, Lopez JD, Deng JG, Coast GM. Enhanced in vivo activity of peptidase-resistant analogs of the insect kinin neuropeptide family. Peptides 2002;23:735–45.
[34] Nassel DR. Neuropeptides in the nervous system of Drosophila and other insects: multiple roles as neuromodulators and neurohormones. Prog Neurobiol 1996;48:325–30.
[35] Pietrantonio PV, Jagge C, Taneja-Bageshwar S, Nachman RJ, Barhoumi R. The mosquito *Aedes aegypti* (L.) leucokinin receptor is a multiligand receptor for the three Aedes kinins. Insect Mol Biol 2005;14:55–67.
[36] Reihle MA, Garczynski SF, Crim JW, Hill CA, Brown MR. Neuropeptides and peptide hormones in *Anopheles gambiae*. Science 2002;298:172–5.
[37] Roberts VA, Nachman RJ, Coast GM, Hariharan M, Chung JS, Holman GM, Williams H, Tainer JA. Consensus chemistry and beta-turn conformation of the active core of the insect kinin neuropeptide family. Chem Biol 1997;4:105–17.
[38] Sadeghi A, Van Damme EJM, Michiels K, Kabera A, Smagghe G. Acute and chronic insecticidal activity of a new mannose-binding lectin from *Allium porrum* against *Acyrthosiphon pisum* via an artificial diet. Can Entomol 2009;141:95–101.
[39] Sadeghi A, Van Damme EJM, Smagghe G. Evaluation of susceptibility of pea aphid to a selection of novel biorational insecticides via artificial diet. J Insect Sci; in press.
[40] Sauvion N, Rahbe Y, Peumans WJ, Van Damme EJM, Gatehouse JA, Gatehouse AMR. Effects of GNA and other mannose binding lectins on development and fecundity of the peach potato aphid *Myzus persicae*. Entomol Exp Appl 1996;79:285–93.
[41] Seinsche A, Dyker H, Losel P, Backhous D, Scherkenbeck J. Effect of helicokinins and ACE inhibitors on water balance and development of *Heliothis virescens* larvae. J Insect Physiol 2000;46:1423–31.
[42] Shakesby AJ, Wallace IS, Isaacs HV, Pritchard J, Roberts DM, Douglas AE. A water-specific aquaporin involved in aphid osmoregulation. Insect Biochem Mol Biol 2009;39:1–10.
[43] Sharma HC, Sharma KK, Crouch JH. Genetic transformation of crops for insect resistance: potentials and limitations. Crit Rev Plant Sci 2004;23:47–72.
[44] Smagghe G, Degheele D. Action of a novel nonsteroidal ecdysteroid mimic, tebufenozide (RH-5992), on insects of different orders. Pestic Sci 1994;42:85–92.
[45] Steer DL, Lew RA, Perlmutter P, Smith AI, Aguilar MI. β-Amino acids: versatile peptidomimetics. Curr Med Chem 2002;2:811–22.

[46] Taneja-Bageshwar S, Strey A, Zubrzak P, Pietrantonio PV, Nachman RJ. Comparative structure-activity analysis of insect kinin core analogs on recombinant kinin receptors from Southern cattle tick *Boophilus microplus* (Acari: Ixodidae) and mosquito *Aedes aegypti* (Diptera: Culicidae). Arch Insect Biochem Physiol 2006;62(3):128–40.
[47] Taneja-Bageshwar S, Strey A, Zubrzak P, Williams H, Reyes-Rangel G, Juaristi E, Pietrantonio PV, Nachman RJ. Identification of selective and non-selective, biostable beta-amino acid agonists of recombinant insect kinin receptors from the southern cattle tick *Boophilus microplus* and mosquito *Aedes aegypti*. Peptides 2008;29:302–9.
[48] Taneja-Bageshwar S, Strey A, Isaac RE, Coast GM, Zubrzak P, Pietrantonio P, Nachman RJ. Biostable agonists that match or exceed activity of native insect kinins on recombinant arthropod GPCRs. Gen Comp Endocrin 2009;162:122–8.
[49] The international aphid genomics consortium. Genome sequence of the pea aphid *Acryrthosiphon pisum*. PLoS Biol; submitted for publication.
[50] Torfs P, Nieto J, Veelaert D, Boon D, van de Water G, Waelkens E, Derua R, Calderon J, de Loof A, Schoofs L. The kinin peptide family in invertebrates. Ann N Y Acad Sci 1999;897:361–73.
[51] Vandenborre G, Van Damme EJM, Smagghe G. Natural products: plant lectins as important tools in controlling pest insects. In: Ishaaya I, Horowitz AR, editors. Biorational control of arthropod pests: application and resistance managements. Springer-Verlag, Dordrecht, Netherlands; in press.
[52] Zubrzak P, Williams H, Coast GM, Isaac RE, Reyes-Rangel G, Juaristi E, Zabracki J, Nachman RJ. Beta-amino acid analogs of an insect neuropeptide feature potent bioactivity and resistance to peptidase hydrolysis. Biopolymers 2007;88(1):76–82.