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Identification of bradykinins in solitary wasp venoms

Katsuhiro Konno[a,b,*], Mario Sergio Palma[a,b], Izaura Yoshico Hitara[b,c], Maria Aparecida Juliano[b,c], Luiz Juliano[b,c], Tadashi Yasuhara[d]

[a]*Center of Study of Social Insects, Department of Biology, Institute of Biosciences of Rio Claro, São Paulo State University, Rio Claro, SP 13506-900, Brazil*

[b]*Center for Applied Toxinology, CEPID/FAPESP, São Paulo, SP 05468-901, Brazil*

[c]*Department of Biophysics, Paulista Medical School, Federal University of São Paulo, São Paulo, SP 04044-020, Brazil*

[d]*Department of Nutrition, Junior College of Agriculture, Tokyo University of Agriculture, Setagaya, Tokyo 156-8502, Japan*

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Abstract

Bradykinins were identified in three solitary wasp venoms. Purification and characterization of the venom extract of the scoliid wasp *Megacampsomeris prismatica* led to the identification of bradykinin and threonine[6]-bradykinin as the major peptide components. The survey of a number of extracts from solitary wasp venom by MALDI–TOF MS revealed that the venoms of two other scoliid wasps, *Campsomeriella annulata annulata* and *Carinoscolia melanosoma fascinata*, also contained Thr[6]-BK as one of the major components. Thus, this study showed the presence of bradykinins in some of the solitary wasp venoms. Moreover, it indicated that these peptides play a major role in their paralyzing action for prey capture because these bradykinins have been shown to block the synaptic transmission of the nicotinic acetylcholine receptor in the insect central nervous system.

Keywords: Bradykinin; Solitary wasp; Venom; MALDI–TOF MS

The venoms of the Hymenoptera are a rich source of various bioactive substances (Piek, 1986). For example, the venoms of the honeybee, hornets and paper wasps have been well documented both chemically and pharmacologically, which revealed that a variety of bioactive peptides and enzymatic proteins act together to produce various biological effects. In contrast, only little is known about the chemical components and biological properties of solitary wasp venoms despite thousands of species inhabiting the planet. Solitary wasps paralyze insects or spiders with their stinging venoms, and feed the paralyzed prey to their larvae. Therefore, the solitary wasp venoms may contain various bioactive substances, in particular, neurotoxins.

The first neurotoxic component characterized in solitary wasp venom was kinins. Piek et al. (1987a,b) isolated threonine[6]-bradykinin (Thr[6]-BK) and megascoliakinin (Thr[6]-BK–Lys–Ala) from the venom of the European scoliid wasp *Megascolia flavifrons* (Yasuhara et al., 1987). Thr[6]-BK was also isolated from the venom of another scoliid wasp *Colpa interrupta* (Piek et al., 1990). These kinins irreversibly block the synaptic transmission of the nicotinic acetylcholine receptor in the insect central nervous system (Piek et al., 1987a,b; Hue and Piek, 1989; Piek, 1991). Shortly thereafter, philanthotoxins were found in the venom of the sphecid wasp *Philanthus triangulum* (Eldefrawi et al., 1988; Piek et al., 1988). They are acylpolyamine toxins and non-competitive antagonists of the postsynaptic glutamate and nicotinic receptors (Nakanishi et al., 1990; Piek and Hue, 1989). We have recently surveyed the bioactive substances in solitary wasp venoms and found novel peptide neurotoxins, pompilidotoxins (PMTXs), from the venoms of the pompilid wasps *Anoplius samariensis* and *Batozonellus maculifrons* (Konno et al., 1997, 1998). PMTXs affect both vertebrate and invertebrate nervous systems, which is due to the slowing or blocking of sodium channel inactivation (Sahara et al., 2000). This activity is similar to those of the α-scorpion and sea anemone toxins, but PMTX can discriminate the neuronal and cardiac sodium channels (Kinoshita et al., 2001). A further survey of solitary wasp venom components led to

* Corresponding author. Tel: +55-19-534-8523; fax: +55-19-534-0009.

E-mail address: kk-gon@rc.unesp.br (K. Konno).

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Table 1
The solitary wasp species examined for the presence of BK and Thr^6-BK in their venoms. The presence of the peaks due to BK (*m/z* 1059.5) and Thr^6-BK (*m/z* 1074.5) were analyzed by MALDI–TOF MS

Solitary wasp species	BK	Thr^6-BK
Pompilidae (pompilid wasps)		
Episyron arrogans	−	−
Anoplius samariensis	−	−
Batozonellus annulatus	−	−
Batozonellus maculifrons	−	−
Tachypompilus analis	−	−
Cyphononyx dorsalis	−	+
Leptodialepis sugiharai	−	−
Eumenidae (eumenid wasps)		
Anterhynchium flavomarginatum micado	−	−
Orancistrocerus drewseni drewseni	−	−
Oreumenes decoratus	−	−
Eumenes fraterculus	−	−
Eumenes micado	−	−
Eumenes rubronotatum rubronotatum	−	−
Eumenes rubrofemoratus	−	−
Sphecidae (sphecid wasps)		
Ammophila subulosa	−	−
Hoplammophila aemulans	−	−
Sceliphron caementarium	−	−
Sphex argentatus argentatus	−	−
Isodontia nigella	−	−
Isodontia harmandi	−	−
Tachytes sinensis sinensis	−	−
Scoliidae (scoliid wasps)		
Megacampsomeris prismatica	+	+
Campsomeriella annulata annulata	−	+
Scolia histrionica japonica	−	−
Scolia decorata ventralis	−	−
Scolia oculata	−	−
Carinoscolia melanosoma fascinata	−	+

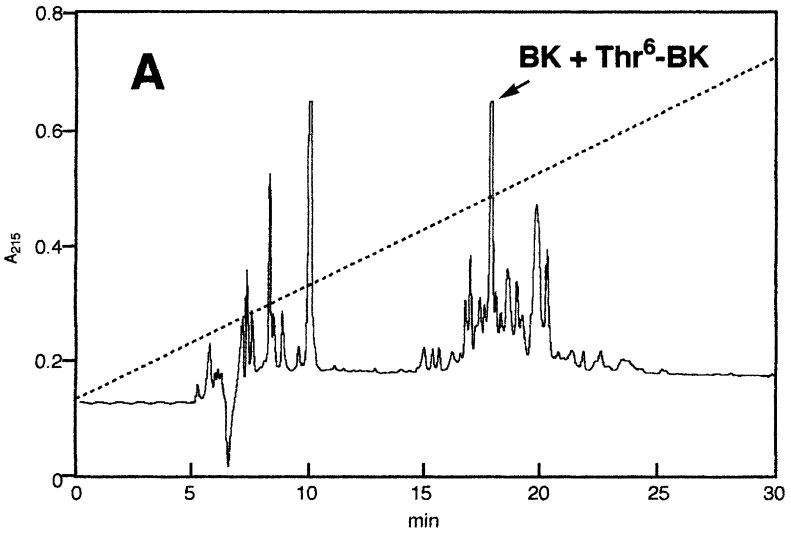
the identification of bradykinins in three scoliid wasp venoms. Since bradykinins have been reported to be neurotoxic in the insect central nervous systems, they may play a major role in their paralyzing action for prey capture. Reported herein are the isolation and identification of these neurotoxic peptides in solitary wasp venoms.

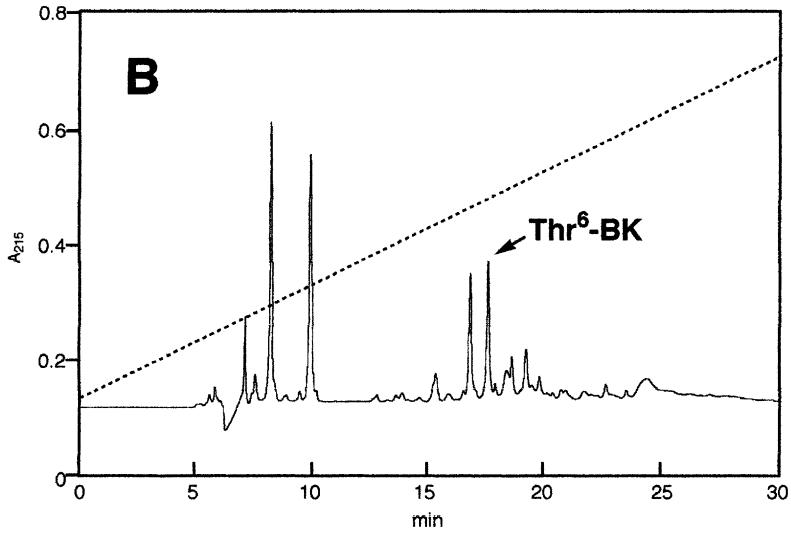
First, bradykinin (BK) and Thr^6-BK were found in the venom extract of the scoliid wasp *Megacampsomeris prismatica*. Twenty-four lyophilized venom sacs of *M. prismatica*, collected in Kanagawa, Ibaraki and Kyoto, Japan, were extracted with 1:1 CH_3CN/H_2O/0.1% TFA (0.2 ml × 4) and the extracts were subjected to reverse-phase HPLC using CAPCELL PAK C_{18}, 10 × 250 mm with a linear gradient from 5 to 65% CH_3CN/H_2O/0.1% TFA over 30 min at a flow rate of 2.5 ml/min (Fig. 1A). The major peak eluted at 17.3 min was collected and further purified by reverse phase HPLC using CAPCELL PAK C_{18}, 6 × 150 mm with isocratic elution of 18% CH_3CN/H_2O/0.1% TFA at flow rate of 1 ml/min. An aliquot of the main fraction eluted at 26.5 min was taken for sequence analysis by Edman degradation. This was shown to be about a 1:2 mixture of BK and Thr^6-BK since the sequence of nine amino acids was shown as Arg–Pro–Pro–Gly–Phe–Ser/Thr–Pro–Phe–Arg, where the 6 position appeared as an approximately 1:2 mixture of Ser and Thr. The MALDI–TOF MS (Micromass TofSpec-2, positive ion mode, α-cyano-4-hydroxycinnamic acid as the matrix) of this fraction was consistent with this result; the peaks at *m/z* 1059.5 and 1074.5 $(M + H)^+$ appeared at about a 1:2 intensity, corresponding to BK and Thr^6-BK, respectively. Comparison of these peptides with the authentic specimens by HPLC finally confirmed the presence of these peptides.

We further surveyed the solitary wasp venoms for the presence of these kinins using MALDI–TOF MS. The venom extracts of 26 species from four families were prepared in the same manner as described above and analyzed by MALDI–TOF MS, 'screening' the peaks due to BK (*m/z* 1059.5) and Thr^6-BK (*m/z*1074.5). The examined species are listed in Table 1. Most of them are dominant species in Japan. As a consequence, the Thr^6-BK peak was found in the extracts from three species, the scoliid wasps *Campsomeriella annulata annulata* and *Carinoscolia melanosoma fascinata* and the spider wasp *Cyphononyx dorsalis*, whereas the BK peak was never found in any venom extract. Of these, the isolation and identification of Thr^6-BK from the venom of the spider wasp *Cyphononyx dorsalis* has already been reported (Konno et al., 2001). The HPLC profiles of the venom extracts from *Campsomeriella annulata annulata* and *Carinoscolia melanosoma fascinata* are shown in Fig. 1B and C, respectively. Purification in the manner similar to that described above, followed by comparison with the synthetic authentic peptide by HPLC confirmed the presence of Thr^6-BK in these venoms. As seen in their HPLC profiles, this peptide is one of the major peptide components in these scoliid wasp venoms.

Bradykinin related peptides have been found in many venoms of Hymenoptera such as ants and social wasps (Nakajima et al., 1985; Piek et al., 1989; Piek, 1991). In particular, those found in the Vespid wasp venoms are collectively called wasp kinins. They have the sequence of BK or Thr^6-BK in their 11–18 amino acid chain and exhibit similar pharmacological activities to BK. When injected into vertebrate predators by stinging, they produce severe pain, thus playing a significant role in their defense system. On the other hand, kinins may play a different role in

Fig. 1. The HPLC profiles of the extracts of the solitary wasp venoms. Each venom extract was injected to the reverse-phase HPLC column using CAPCELL PAK C_{18}, 10 × 250 mm, with a linear gradient from 5 to 65% Me_3CN–H_2O containing 0.1% TFA for 30 min, monitored by UV 214 nm. (A) *Megacampsomeris prismatica*, (B) *Campsomeriella annulata annulata*, (C) *Carinoscolia melanosoma fascinata*.


A
BK + Thr6-BK
A215
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
min


B
Thr6-BK
A215
0.8
0.6
0.4
0.2
0
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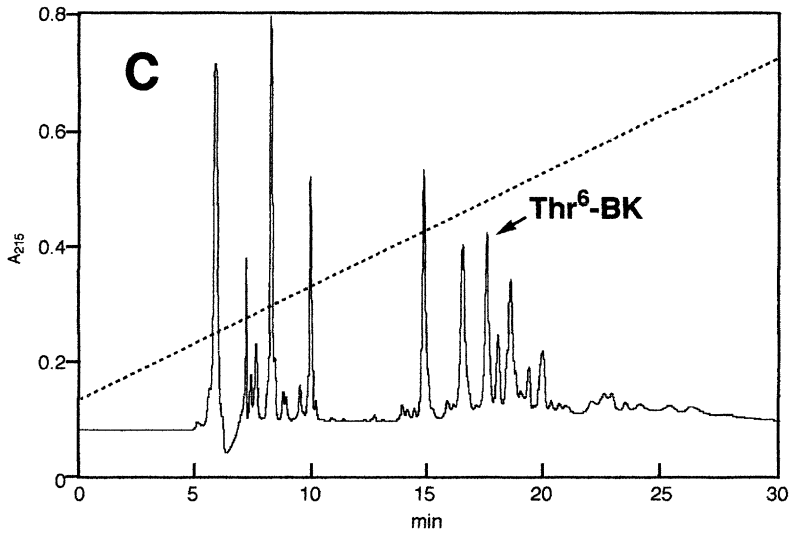
C
Thr6-BK
A215
0.8
0.6
0.4
0.2
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10
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min

solitary wasp venoms. Piek and co-workers isolated Thr^6-BK and megascoliakinin (Thr^6-BK–Lys–Ala) from European scoliid wasp venoms (Piek et al., 1987a,b; Yasuhara et al. 1987), and investigated the effects of these peptides on the insect central nervous system because scoliid wasps use their venom to paralyze beetle larvae by stinging them in the nerve ganglia. These kinins irreversibly block the nicotinic synaptic transmission from the cercal nerve to a giant interneuron in the sixth abdominal ganglion of the cockroach *Periplaneta americana* by depletion of the transmitter in the presynaptic site (Piek et al., 1987a,b). These results indicated that the kinins, in particular Thr^6-BK, may be the most important toxin in these venoms, causing irreversible paralysis in prey (Hue and Piek, 1989; Piek et al., 1990; Piek, 1991). Piek et al. also examined the presence of kinins in a variety of Hymenopteran venoms by pharmacological means, and found kinin-like activity in the venoms of the scoliid, tiphiid and mutillid wasps (Piek et al., 1989; Piek, 1991).

This study proved the presence of bradykinins in some of the scoliid wasp venoms, and indicated that these peptides play a major role in their paralyzing action for prey capture. In contrast to the scoliid wasps, bradykinins were not found in the eumenid and sphecid wasp venoms. However, it does not imply that these solitary wasp venoms do not contain any kinin and its related peptides since this study focused only on BK and Thr^6-BK. Searching for other kinins in solitary wasp venoms may be of great interest.

It is noteworthy that MALDI–TOF MS is a useful means for the rapid 'screening' of particular target compounds in a number of venom extracts with minute amount.

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