Short communication

Isolation and sequence determination of peptides in the venom of the spider wasp (Cyphononyx dorsalis) guided by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry

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Abstract

Micro-scale (sub-pmol) isolation and sequence determination of three peptides from the venom of the solitary spider wasp Cyphononyx dorsalis is described. We isolated two novel peptides Cd-125 and Cd-146 and a known peptide Thr 6-bradykinin from only two venom sacs of solitary spider wasp Cyphononyx dorsalis without bioassay-guided fractionation, but instead guided by MALDI-TOF MS. The MALDI-TOF MS analysis of each fraction showed the purity and molecular weight of the components, which led to the isolation of the peptides virtually without loss of sample amount. The sequences of the novel peptides Cd-125 (Asp-Thr-Ala-Arg-Leu-Lys-Trp-His) and Cd-146 (Ser-Glu-Thr-Gly-Asn-Thr-Val-Thr-Lys-Gly-Phe-Ser-Pro-Leu-Arg) were determined by Edman degradation together with mass spectrometry, and finally corroborated by solid-phase synthesis. The known peptide Thr 6-bradykinin (Arg-Pro-Pro-Gly-Phe-Thr-Pro-Phe-Arg) was identified by comparison with the synthetic authentic specimen. This is the first example for any kinins to be found in Pompilidae wasp venoms. The procedure reported here can be applicable to studies on many other components of solitary wasp venoms with limited sample availability. © 2001 Elsevier Science Ltd. All rights reserved.

Arthropod venoms have attracted much attention for years as a rich source of bioactive substances, in particular of neurotoxins. A number of toxins isolated from scorpion, spider and wasp venoms have been extensively used as research tools for neuronal functions, such as elucidating action mechanisms of ion channels or characterizing receptor functions, and some of them are of clinical interest for neurological disorders (Harvey et al., 1993).

Solitary wasps can be included among those arthropods since they inject their venoms into insects or spiders and paralyze them, and hence, the venoms may contain a variety of neurotoxins (Piek and Spanjer, 1986). Compared to scorpions and spiders, however, only a few solitary wasp venoms have been studied chemically despite thousands of species inhabiting the planet. Philanthotoxins are acylpolyamines in the venom of the digger wasp Philanthus triangulum, which are non-competitive antagonists of glutamate and nicotinic receptors (Eldefrawi et al., 1988; Piek et al., 1988). The venoms of the scolid wasps Megascolia flavifrons and Colpa interrupta contain peptides similar to bradykinin, which presynaptically block nicotinic acetylcholine receptors in the insect central nervous system (Piek et al., 1990; Yasuhara et al., 1987).
We have recently found novel peptide neurotoxins, pompilidotoxins (PMTXs), from the venoms of the spider wasps *Anoplius samariensis* and *Batozonellus maculifrons* (Konno et al., 1998). PMTXs facilitate neurotransmission both at invertebrate neuromuscular junctions and in mammalian central nervous systems, which is due to slowing or blockade of Na⁺ channel inactivation (Sahara et al., 2000). We also found a new mast cell degranulating peptide eumenine mastoparan-AF (EMP-AF) in the venom of the eumenine wasp *Anterhynchium flavomarginatum micado* (Konno et al., 2000).

In order to isolate and characterize toxins in solitary wasp venoms by bioassay-guided fractionation in the usual manner, it is necessary to collect a large number of wasps. Usually, however, collecting such a large number is extremely difficult. Therefore, micro-bioassay and/or micro-scale isolation and chemical characterization is necessary. Micro-perfusion of smooth muscles was used for detecting kinin activity in wasp and ant venoms (Piek, 1991). In the case of scorpion venoms, a microinjection technique using *Drosophila melanogaster* for bioassay-guided isolation of neurotoxins was reported, demonstrating its advantage when limited amounts of material are available (Escoubas et al., 1995). For spider toxins, new highly sensitive analytical methods using LC–MS and tandem mass spectrometry (MS/MS) have been recently developed, which made it possible to detect and characterize up to 40 acylpolyamines from a single venom gland (Itagaki et al., 1997; Palma et al., 1997). Quite recently we applied collision induced dissociation/post-source decay (CID/PSD) with matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) to sequence novel peptides from solitary wasp venoms, and proved it useful for sequencing unknown peptides with a limited amount of venom supply (Hisada et al., 2000). These results prompted us to isolate solitary wasp toxins from a limited number of venoms without bioassay-guided fractionation, but instead guided by MALDI-TOF MS. The MALDI-TOF MS analysis of each fraction revealed the purity and molecular weight of the components, which led to the isolation of the peptides virtually without loss of sample amount. We report here the isolation and sequence determination of three peptides starting from only two venom sacs of the spider wasp *Cyphononyx dorsalis* by MALDI-TOF MS-guided fractionation.

Two female wasps of *C. dorsalis* collected in Kyoto, Japan in August 1998 were immediately frozen by dry ice and stored at −75°C. The venom sacs were dissected from freshly thawed abdomens, lyophilized and kept at −20°C until use. The lyophilized venom sacs were extracted with 1:1 acetonitrile–water containing 0.1% trifluoroacetic acid (CH₃CN/H₂O/0.1% TFA). MALDI-TOF MS analysis of a small aliquot of the crude extracts gave the major peaks at m/z 1026, 1075, 1244 and 1693 due to molecular ion peaks (M + H)⁺ (Fig. 1), indicating that most of the venom components were peptides. The extracts were fractionated by a reverse-phase HPLC (Fig. 2) without any further treatment, and each fraction was analyzed by MALDI-TOF MS. The main fraction eluted at 15 min showed the major MS peak at m/z 1026 along with some other small peaks.

![Fig. 1. MALDI-TOF MS of the crude venom extract from the spider wasp Cyphononyx dorsalis.](image-url)
Accordingly, this fraction was further purified by a reverse-
phase HPLC in isocratic conditions to isolate the peptide
Cd-125, showing high purity with MALDI-TOF MS, the
molecular ion peak at $m/z$ 1026 [(M + H)$^+$, monoisotopic].
Edman degradation using 1/10 of the whole amount gave a
sequence of eight amino acids as Asp-Thr-Ala-Arg-Leu-
Gln-Trp-His. Both the MS peak at $m/z$ 1026 (M + H)$^+$ and
MALDI-TOF MS/MS analysis were consistent with
this sequence, which was finally corroborated by compar-
ison of HPLC retention time and MS data with the synthetic
specimen obtained by solid-phase synthesis. Thus, the struc-
ture of Cd-125 was unambiguously determined as Asp-Thr-
Ala-Arg-Leu-Gln-Trp-His. This sequence showed no
homology to any other known peptide and, therefore, is a
novel peptide. Effects of this peptide on various vertebrate
and invertebrate nervous systems, including lobster neuro-
muscular transmission, for which PMTX shows a powerful
facilitatory action (Konno et al., 1998), are under investiga-
tion using the synthetic specimen.

The minor fraction eluted at about 17 min was puri-
fied further by reverse phase HPLC in isocratic conditions to
isolate the minor peptide Cd-146 with the MS peak at $m/z$
1693 (M + H)$^+$ and MALDI-TOF MS/MS analysis were consistent with
this sequence, which was finally corroborated by compar-
ison of HPLC retention time and MS data with the synthetic
specimen obtained by solid-phase synthesis. This is also a novel peptide, but the C-terminal six residue
sequence is quite similar to that of bradykinin, suggesting
that it may be a wasp kinin-type peptide. Preliminary tests
using the synthetic specimen of this peptide showed clear
hyperalgesic effect on rats without any sign of inflamma-
tion. Further investigation in more detail is in progress.

The quite minor fraction eluted after the Cd-146 fraction
at 17.5 min showed an MS peak at $m/z$ 1075 (M + H)$^+$.
This was coincident with that of Thr$^6$-bradykinin (Thr$^6$-BK)
isolated previously from scoliid wasps (Piek et al., 1990;
Yasuhrara et al., 1987). Edman degradation using 1/3 of
the whole amount proved the sequence (Arg-Pro-Pro-Gly-
Phe-Thr-Pro-Phe-Arg), which was finally confirmed by
comparison of HPLC retention time and MS data with the
synthetic authentic specimen. Piek et al. (1990) reported that
Thr$^6$-BK may be the most important toxin in the venoms of
the European scoliid wasps, causing irreversible paralysis in
prey probably by depletion of transmitter acetylcholine in
the nerve terminal. They also surveyed Hymenopteran
venoms for the presence of kinins. As for solitary wasps,
kinins were found in three families, Scoliidae, Tiphidiidae and
Mutillidae, but not in the family Pompilidae (Piek, 1991).
Accordingly, this is the first example for any kinins to be
found in Pompilidae wasp venoms.

Additionally, adenosine was identified in the fraction
eluted at 8.5 min. The MS peak at $m/z$ 268 (M + H)$^+$ and
the UV and $^1$H NMR spectra were consistent with those of
adenosine, which was confirmed by comparison of HPLC
retention time with authentic specimen.

In this study, we have isolated and characterized three

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Fig. 2. Fractionation of the venom extract of the spider wasp Cyphononyx dorsalis by reverse-phase HPLC using CAPCELL PAK C$_{18}$
(10 × 250 mm) with linear gradient of 5–65% CH$_3$CN/H$_2$O/0.1% TFA over 30 min at flow rate of 2.5 ml/min. UV absorption was monitored
at 215 nm.
peptides starting from only two venom sacs of the spider wasp *Cyphononyx dorsalis*, demonstrating the utility of MALDI-TOF MS-guided fractionation for micro-scale isolation. The procedure described here is applicable to isolation of many other solitary wasp toxins since its sample availability is quite limited. If the chemical structure was fully characterized, synthesis can provide sufficient amount of sample for biological studies. In the case of simple peptides such as Cd-125 and Cd-146, they are readily available by solid-phase automated synthesis. Studies on biological properties of these novel peptides using synthetic specimens are in progress and the results will be reported in due course.

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References


