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# A naturally-occurring carboxyl-terminally truncated α-scorpion toxin is a blocker of sodium channels

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ABSTRACT

α-Scorpion toxins constitute a multigene family of evolutionarily conserved venom peptides that inhibit sodium channel inactivation and increase its peak current. Here, we describe the characterization of a new α-scorpion toxin gene expressed in the venom gland of *Mesobuthus eupeus* that encodes a carboxyl-terminally truncated product of 38 residues (named MeuNaTxα(NT)-1). Synthetic MeuNaTxα(NT)-1 was oxidized to form two disulfide bridges in an alkaline environment and the refolded peptide exhibits different structure and function from the classical α-scorpion toxin. MeuNaTxα(NT)-1 blocks sodium channels on rat dorsal root ganglia (DRG) neurons without impact on the inactivation of the channels. This work provides a clue for evolution-guided design of channel blockers for therapeutic aims.



## 1. Introduction

Voltage-gated sodium channels (VGSCs) are crucial players of intrinsic neuronal, muscle and cardiac excitability. They are large, complex membrane proteins composed of an α-subunit of approximately 260 kDa associated with one or more accessory β-subunits. The α-subunit is composed of four homologous domains (DI–DIV) connected by intracellular and extracellular loops, each domain containing six helical transmembrane segments (S1–S6), and a hairpin-like P loop between S5 and S6 that forms the ion selectivity filter [1,2]. In the α-subunits, there are at least seven distinct receptor sites identified for various neurotoxins, where sites 3, 4 and 6 are targeted by several types of water-soluble polypeptide toxins from scorpions, spiders, sea anemone, and cone snails. Site 1 located at the extracellular pore opening of the α-subunits is shared by the peptide toxin μ-conotoxins from cone snails and tetrodotoxin (TTX) [3,4], a classical VGSC blocker isolated from diverse venomous species, which distinguish VGSCs into TTX-resistant (TTX-R) and TTX-sensitive (TTX-S) sodium channels [5]. It has long been known that VGSC blockers targeting site 1 are anaesthetics and analgesics and these blockers are also useful in treating cardiac heart arrhythmias and epilepsy [6–8].

Scorpion toxins affecting VGSCs constitute a family of evolutionarily conserved gating modulators composed of 61–76 residues [9]. They possess a common cysteine-stabilized α-helical and β-sheet (CSαβ) structural core with four disulfide bridges [10]. According to different pharmacological activities and binding features, these toxins can be classified into two distinct functional groups, α and β [11–14]. The α-toxins slow the sodium channel inactivation to induce prolongation of action potentials by binding to site 3, whereas β-toxins modify the activation process of channels by binding to site 4. In this work, we describe for the first time a naturally-occurring carboxyl-terminally truncated α-toxin that functions as a blocker of VGSCs rather than a gating modifier of these channels.

## 2. Materials and methods

### 2.1. Chemical synthesis and oxidative refolding

The reduced MeuNaTxα(NT)-1 was chemically synthesized by ChinaPeptides Co., Ltd. (Shanghai, China). For oxidative refolding to form two disulfide bridges, the peptide was dissolved in 0.1 M Tris–HCl buffer (pH 8.0) to a final concentration of 1 mM and incubated at 25 °C for 48 h. The peptide was purified to homogeneity by reversed-phase high-pressure liquid chromatography (RP-HPLC). Purity and molecular mass of MeuNaTxα(NT)-1 was

*Abbreviations:* 3′-UTR, 3′-untranslational region; CD, circular dichroism; DRG, dorsal root ganglia; $IC_{50}$, 50% inhibitory concentration; VGPC, voltage-gated potassium channel; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; NMD, nonsense-mediated mRNA decay; NMR, nuclear magnetic resonance; ORF, open reading frame; PSC, premature stop codon; RP-HPLC, reversed-phase high-pressure liquid chromatography; $T_R$, retention time; TTX, tetrodotoxin; VGSC, voltage-gated sodium channel.

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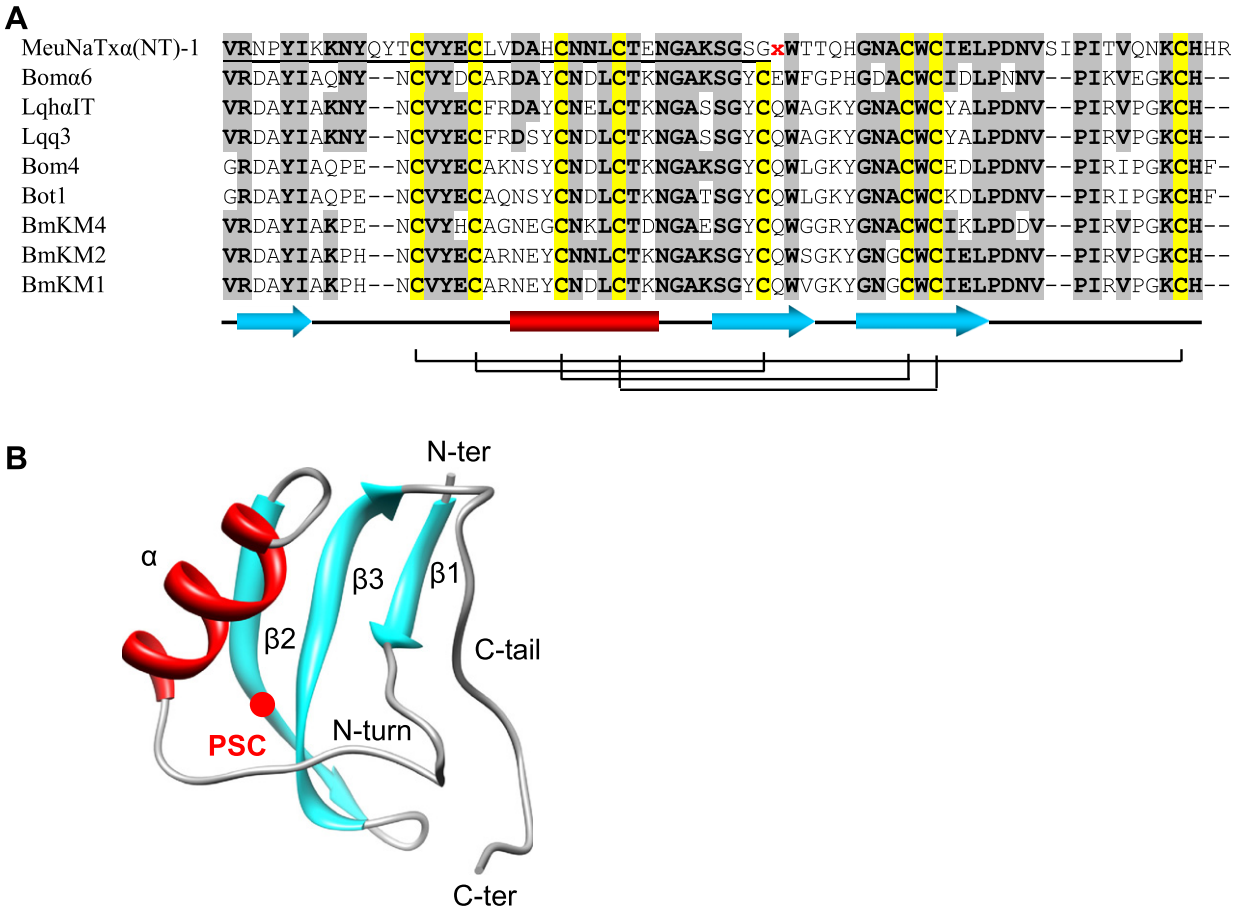


**Fig. 1.** MeuNaTxα(NT)-1 is a truncated analogue of α-scorpion toxins. "x" in red represents PSC. The mature MeuNaTxα(NT)-1 for chemical synthesis is underlined once. Cysteines for involving in the formation of disulfide bridges are shadowed in yellow and residues identical to those of MeuNaTxα(NT)-1 and -2 in gray. Secondary structure elements of BmKM1 [15,16] were extracted from its experimental structure (PDB code 1SN1) by STRIDE (http://webclu.bio.wzw.tum.de/stride/). Four conserved disulfide bridges are indicated by lines; (B) Ribbon structure of BmKM1 prepared by Chimera (http://www.cgl.ucsf.edu/chimera/), showing the location of the MeuNaTxα(NT)-1 PSC in a structural context. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

determined by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) on a Kratos PC Axima CFR plus (Shimadzu Co., Ltd., Kyoto, Japan).

### 2.2. Circular dichroism (CD) spectroscopy

CD spectra of the refolded MeuNaTxα(NT)-1 and BmKM1, a typical α-scorpion toxin isolated from the venom of *Mesobuthus martensii* [10,15,16] were recorded on a JASCO J-720 spectropolarimeter (Tokyo, Japan) at a protein concentration of 0.3 mg/ml dissolved in water. Spectra were measured at 20 °C from 240 to 195 nm by using a quartz cell of 1.0 mm thickness and data were collected at 0.5 nm intervals with a scan rate of 50 nm/min. The measurement of CD spectra was performed by averaging three scans. Data are expressed as mean residue molar ellipticity ($[\theta]$).

### 2.3. Electrophysiological assays for DRG neurons

The method for isolating dorsal root ganglia (DRG) neurons from SD rats (100–150 g) has been described previously [17]. Sodium currents from these cells were recorded by the whole cell patch clamp recording technique. Resistances of micropipettes used here were 3.0–6.0 MΩ after filled with the internal solution containing (in mM): CsF 135, NaCl 10, HEPES 5, adjusted to pH 7.0 with 1 M CsOH. The external bathing solution contains (in mM): NaCl 30, KCl 5, CsCl 5, $MgCl_2$ 1, $CaCl_2$ 1.8, HEPES 5, TEA-Cl 90, D-glucose 25, adjusted to pH 7.4 with NaOH. Membrane currents recordings were made by an AxoClamp 700A amplifier (Axon Inc.) and DIGIDATA 1322A (Axon Inc.). Pulse stimulation and data acquisition were controlled by Clampex 9.0 software (Axon Inc.). 200 nM TTX was used to separate TTX-R from TTX-S sodium currents.

### 2.4. Comparative modeling

To obtain templates with a similar cysteine pattern to MeuNaTxα(NT)-1 for comparative modeling, we searched the structural database (http://www.expasy.org/) by ScanProsite, in which the pattern C-x(3)-C-x(4,5)-C-x(3)-C derived from MeuNaTxα(NT)-1 was used as query. Once a reliable alignment between MeuNaTxα(NT)-1 and a template is available, comparative modeling is performed by Homer (http://protein.cribi.unipd.it/homer/).

## 3. Results

### 3.1. MeuNaTxα(NT)-1 is a carboxyl-terminally truncated α-scorpion toxin

In an attempt to isolate new α-scorpion toxin cDNA clones from the venom gland of *Mesobuthus eupeus*, we unexpectedly cloned an unusual cDNA of 271 bp that encodes a typical α-scorpion toxin with a premature stop codon (PSC) at site 39 which results in a C-terminally truncated peptide of 38 residues named MeuNaTxα(NT)-1 (accession number: HM 989915). When the PSC is