OD1, the first toxin isolated from the venom of the scorpion *Odonthobuthus doriae* active on voltage-gated Na^+ channels

Amir Jalali[a,1], Frank Bosmans[b,1], Mehriar Amininasab[c], Elke Clynen[d], Eva Cuypers[b], Abbas Zaremirakabadi[e], Mohammad-Nabi Sarbolouki[c], Liliane Schoofs[d], Hossein Vatanpour[a], Jan Tytgat[b,*]

[a] *Department of Toxicology and Pharmacology, Shaheed Beheshti University of Medical Science, Tehran, Iran*
[b] *Laboratory of Toxicology, University of Leuven, Naamsestraat 59, B-3000 Leuven, Belgium*
[c] *Institute of Biochemistry and Biophysics, University of Tehran, Iran*
[d] *Laboratory for Developmental Physiology and Molecular Biology, University of Leuven, Naamsestraat 59, B-3000 Leuven, Belgium*
[e] *Razi Vaccine and Serum Research Institute, Hessarak Karaj, Iran*

Received 9 June 2005; revised 20 June 2005; accepted 20 June 2005

Available online 14 July 2005

Edited by Dr. Maurice Montal

Abstract In this study, we isolated and pharmacologically characterized the first α-like toxin from the venom of the scarcely studied Iranian scorpion *Odonthobuthus doriae*. The toxin was termed OD1 and its primary sequence was determined: GVRDAYIADDKNCVYTCASNGYCNTECTKNGAESGYCQWIGRYGNACWCIKLPDEVPIRIPGKCR. Using the two-electrode voltage clamp technique, the pharmacological effects of OD1 were studied on three cloned voltage-gated Na^+ channels expressed in *Xenopus laevis* oocytes ($Na_v1.2/\beta_1$, $Na_v1.5/\beta_1$, para/tipE). The inactivation process of the insect channel, para/tipE, was severely hampered by 200 nM of OD1 (EC_{50} = 80 ± 14 nM) while $Na_v1.2/\beta_1$ still was not affected at concentrations up to 5 μM. $Na_v1.5/\beta_1$ was influenced at micromolar concentrations.

Keywords: Iranian scorpion *Odonthobuthus doriae*; α-like toxin; Voltage-gated Na^+ channel

1. Introduction

Voltage-gated Na^+ channels (VGSCs) are membrane spanning proteins responsible for action potentials in neurons and most excitable cells [1]. Until this date nine mammalian ($Na_v1.1$–$Na_v1.9$) and three insect VGSCs have been cloned [2,3]. Since VGSCs play an important physiological role in vertebrates and invertebrates, they are targeted by toxins from animal venoms and plants [4].

Most scorpion neurotoxins targeting VGSCs are single chain polypeptides composed of 60–70 amino acids cross-linked by 4-disulfide bridges [5–7]. These polypeptides comprise two main groups: α- and β-toxins [8–10]. Scorpion α-toxins bind to site 3 and prolong the action potential by slowing the inactivation of VGSCs. According to their different pharmacological and binding properties, the α-toxins can be further divided into three subgroups, classical α-, α-like and insect α-toxins. Classical α-toxins (e.g., AaHII, LqhII) are highly toxic to mammals, whereas the insect α-toxins (e.g., LqhαIT) are highly toxic to insects. The more recently characterized α-like toxins (e.g., BmK M1, LqhIII) act on both mammals and insects, but are unique in their inability to bind to rat synaptosomes despite a high toxicity by intravenous injection [8,11–14].

The Iranian yellow scorpion *Odonthobuthus doriae* is a member of the Buthidae family. The genus Buthus, with the two species *doriae* and *odonturus*, are endemic to the Old World, mainly the Middle East [7,15]. Specifically, the scorpion described here can be found in the central and southern part of Iran. Its sting can cause various effects ranging from local pain, inflammation and necrosis to muscle paralysis and hematuria. Since very little is published about this scorpion and the bioactive substances in its venom, we tried to determine whether VGSC toxins were present.

As a consequence, we report here the isolation and pharmacological characterization of OD1, the first α-like toxin from the venom of the Iranian yellow scorpion *O. doriae* scorpion.

2. Materials and methods

2.1. Purification

500 mg of the crude venom (gift from Dr. Akbary; Vaccine and Serum production and Research Institute of Razi, Karaj, Iran) was dissolved in 10 ml of mobile phase (100 mM ammonium acetate in water, pH 6.8) (Merck, Germany). After sample clarification by centrifugation, fractionation was carried out using two 210 × 3.5 cm Sephadex® G_{50} columns in series (flow rate: 1 ml/min) (Pharmacia, Sweden) at room temperature (20–22 °C) [16,17]. Absorbance was measured at 280 nm. Toxicity guided separation of various protein fractions was accomplished after i.v. injection into mice (white, male, 18–20 g). Fraction 4, containing the toxin, was further purified by ion exchange chromatography (Pharmacia) using a 2.5 × 15 cm column (elution buffers: Tris 50 mM at pH 8 (solution A) and Tris 50 mM at pH 8 plus 1 M NaCl (Solution B)). The first equilibrium step of 60 ml of solution A was followed by a gradient from solution A to solution B (240 ml). The flow rate was 2 ml/min and the absorbance was measured at 280 nm.

*Corresponding author. Fax: +3216323405.
E-mail address: Jan.Tytgat@pharm.kuleuven.be (J. Tytgat).

URL: http://www.toxicology.be.

[1] Both authors contributed equally.

Abbreviations: DTT, dithiothreitol; MS, mass spectrometry; RP-HPLC, reversed-phase high performance liquid chromatography; TEVC, two-electrode voltage clamp; TFA, trifluoro acetic acid; VGSC(s), voltage-gated Na^+ channel(s)

0014-5793/$30.00
doi:10.1016/j.febslet.2005.06.052

Lyophilized fraction 3 of the ion exchange chromatography was further purified on a semi-preparative reversed-phase high performance liquid chromatography (RP-HPLC) Vydac® C_8 column (250 × 10 mm, 5 μm) (Hesperia, CA, USA) equilibrated with 0.1% w/v trifluoro acetic acid (TFA). Elution was performed using a linear gradient (20–30%) of acetonitrile containing 0.1% w/v TFA in 45 min (flow rate 3 ml/min). Absorbance was recorded at 215 nm. The final purity of the toxin was assessed on an analytical Supelco® RP-HPLC column (250 × 4.6 mm, 5 μm) using solution A (0.1% TFA in water) and solution B (0.1% TFA in acetonitrile) with a gradient up till 60% B over a period of 45 min (SMART®, Pharmacia). Flow rate and absorbance were 500 μl/min and 214 nm, respectively.

2.2. Mass spectrometry

The molecular mass of OD1 and fragments generated by enzymatic cleavage were determined by MALDI-TOF mass spectrometry (MS) on a Reflex IV (Bruker Daltonics GmbH, Germany) using a recrystal-

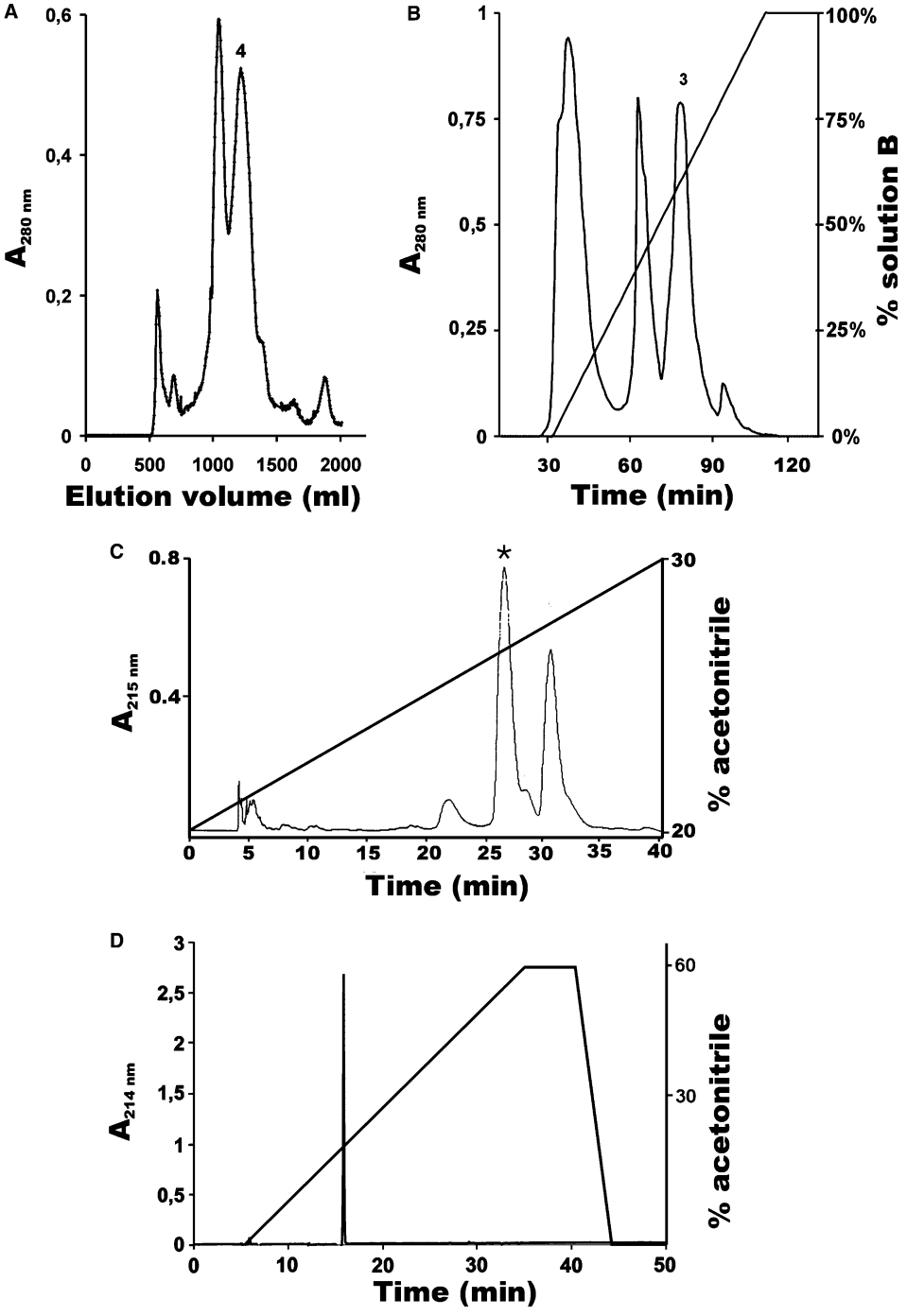

Fig. 1. Purification of OD1 from the venom of *O. doriae*. (A) Crude venom (500 mg) was dialyzed and 107 mg was fractionated by gel filtration. Toxic fraction 4 was recovered and dried. (B) Ion exchange chromatography of fraction 4 (9 mg) obtained in (A). Solution B consists of Tris 50 mM (pH 8) plus 1 M NaCl. (C) Fraction 3 (2 mg) of the ion exchange step was further purified using a C_8 column. Fractions eluting at 26.9 min (*) were recovered and dried (800 μg). (D) The purity of the toxin was assessed on an analytical RP-HPLC C_{18} column. OD1 encompasses about 1% of the total venom.