Toxicon 119 (2016) 274–279

Contents lists available at ScienceDirect

Toxicon

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

Short communication

Central loop of non-conventional toxin WTX from *Naja kaouthia* is important for interaction with nicotinic acetylcholine receptors

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ARTICLE INFO

Article history:
Received 15 April 2016
Received in revised form
9 June 2016
Accepted 21 June 2016
Available online 23 June 2016

Keywords:
Three-finger protein
Snake neurotoxin
Ly-6/uPAR
Site-directed mutagenesis
Nicotinic acetylcholine receptor
Computer modeling

ABSTRACT

'Three-finger' toxin WTX from *Naja kaouthia* interacts with nicotinic and muscarinic acetylcholine receptors (nAChRs and mAChRs). Mutagenesis and competition experiments with ^{125}I-α-bungarotoxin revealed that Arg31 and Arg32 residues from the WTX loop II are important for binding to *Torpedo californica* and human α7 nAChRs. Computer modeling suggested that loop II occupies the orthosteric binding site at α7 nAChR. The similar toxin interface was previously described as a major determinant of allosteric interactions with mAChRs.

The major components of snake venoms are 'three-finger' toxins – the small β-structural proteins (60–75 a.a.) which consist of three loops ('fingers') protruding from the compact globular core ('head') stabilized by four invariant disulfide bonds. Three-finger toxins act on numerous targets (Kini and Doley, 2010). Curaremimetic 'three-finger' toxins targeting nicotinic acetylcholine receptors (nAChRs, pentameric ligand-gated ion channels) can be subdivided into three classes: short-chain and long-chain α-neurotoxins (α-NTs), and non-conventional neurotoxins (Fig. 1A). Short-chain (4 disulfide bonds, 60–62 a.a.) and long-chain (5 disulfide bonds, 66–75 a.a.) α-NTs selectively inhibit muscle-type nAChRs with typical affinity in nanomolar range, while long-chain α-NTs with additional disulfide bond located in the tip of the central loop (loop II) interact with both muscle and neuronal α7 nAChRs (Mourier et al., 2000). Unlike long-chain α-NTs, the non-conventional neurotoxins contain additional 5th disulfide bond in the *N*-terminal loop (loop I) (Kini and Doley, 2010). Most of the non-conventional toxins are characterized by a low level of toxicity (LD_{50} – 5–80 mg/kg) which is significantly less than that of short-chain and long-chain α-NTs (LD_{50} – 0.04–0.30 mg/kg) (Nirthanan et al., 2003). However, some of non-conventional toxins are highly toxic, e.g. candoxin from *Bungarus candidus* (Cndx, LD_{50} ~ 0.83 mg/kg) (Nirthanan et al., 2003) and γ-bungarotoxin from *Bungarus multicinctus* (γ-Bgtx, LD_{50} ~ 0.15 mg/kg) (Aird et al., 1999).

Non-conventional 'weak' toxin from *Naja kaouthia* (WTX) is non-lethal to mice at doses up to 2 mg/kg (Mordvintsev et al., 2007) and shares some biological properties of α-NTs acting on nAChRs and three-finger muscarinic toxins (MTs) acting on muscarinic acetylcholine receptors (mAChRs, G-protein coupled receptor family). It binds irreversibly to muscle and α7 nAChRs with IC_{50} in micromolar range (Utkin et al., 2001a) and allosterically interacts with different subtypes of mAChRs (Mordvintsev et al., 2009). Recently, site-directed mutagenesis and computer modeling

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http://dx.doi.org/10.1016/j.toxicon.2016.06.012
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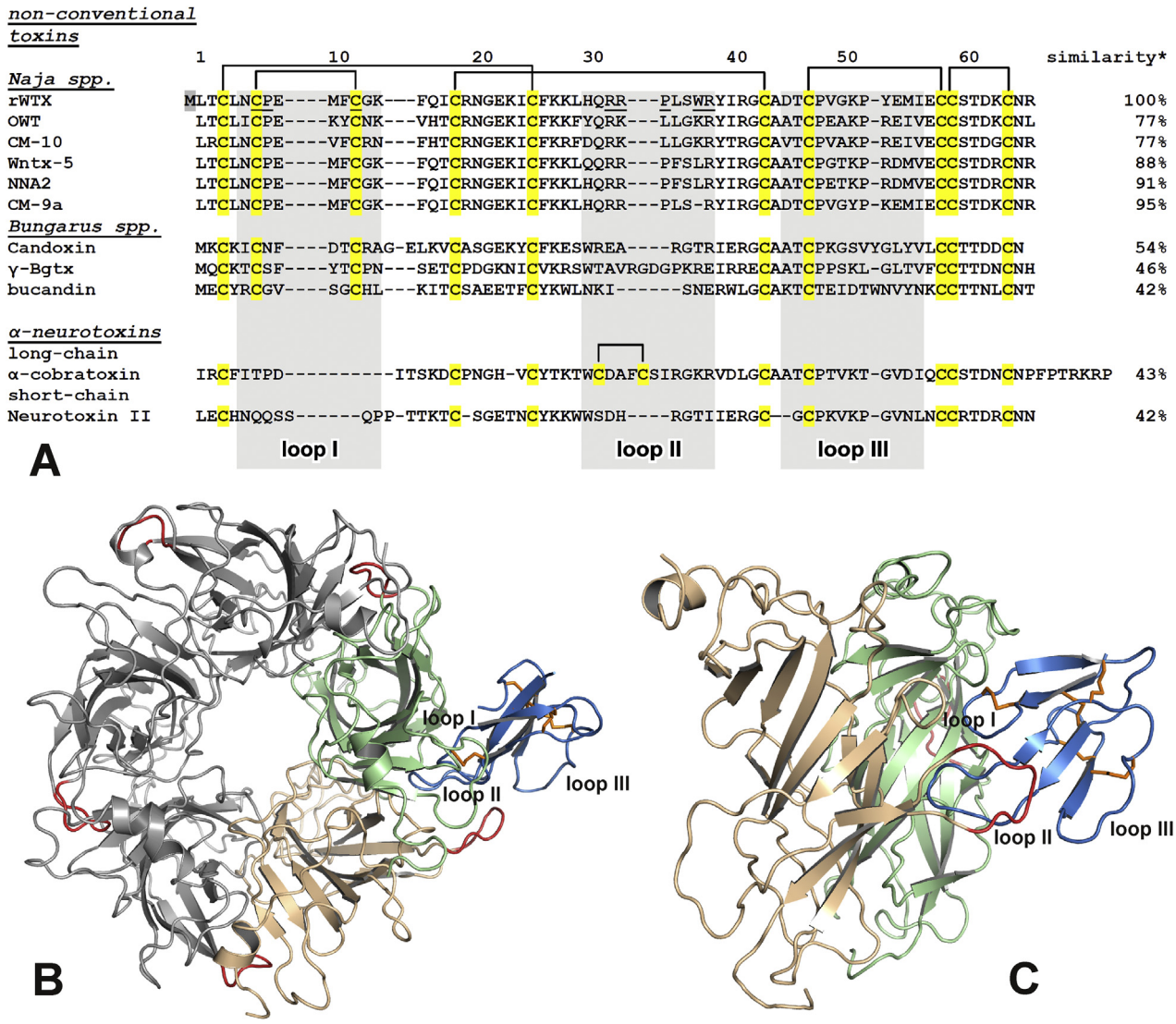

Fig. 1. (A). Alignment of amino acid sequences of rWTX, non-conventional toxins from *Naja* and *Bungarus* species, and α-neurotoxins. Cysteines and position of the loops in the 'three finger' scaffold are highlighted by the yellow and grey background, respectively. Sites of point mutations introduced into the rWTX molecule are underlined. Additional *N*-terminal Met residue in the rWTX molecule is shown. The sequence similarity between WTX and other proteins was calculated by EMBOSS Stretcher (EMBL-EBI). (B,C). Top and side views of the complex between the rWTX[P33A] mutant toxin and the 'open' orthosteric site at the extracellular ligand-binding domain of α7 nAChR (solution from the cluster #1, see text for details). Toxin molecule is in *blue* and its disulfide bonds are in *orange*. The three loops of the toxin are labeled. The principal (+) and complementary (−) subunits of the homopentameric α7 receptor are in *wheat* and *light green*, respectively. The loop C of each receptor subunit is colored in *red*.

revealed that the positively charged residues from the tip of the toxin's loop II (Arg31, Arg32, and Arg37) are important structural determinants of WTX interaction with human M1 and M3 mAChRs (Lyukmanova et al., 2015). NMR study of the WTX structure and dynamics pointed to the high conformational plasticity of the toxin's loop II. We proposed that this property underlies the ability of WTX to interact both with nAChRs and mAChRs (Lyukmanova et al., 2015). The importance of the flexibility of the three-finger scaffold for interaction with different molecular targets was previously discussed on the example of chimaeras of acetylcholinesterase inhibitor fasciculin 2 and short-chain toxin α (Ricciardi et al., 2000).

To verify our hypothesis and to study the influence of other WTX structural features on its ability to interact with nAChRs, in the current work we analyzed the activity of ten mutant variants of the toxin (Fig. 2). The recombinant toxin (rWTX) and all its mutants were produced using previously developed bacterial expression system (Lyukmanova et al., 2009). The recombinant toxins contain additional *N*-terminal Met residue appearing due to translation of starting atg codon. The following rationales were used for the design of WTX mutants. (1) The main feature of non-conventional toxins is the 5th disulfide in the loop I. To investigate its functional relevance we 'disrupted' this bond replacing corresponding Cys6 and Cys11 residues by Ser. (2) WTX demonstrates a conformational heterogeneity in solution due to the *cis-trans*-isomerization of the peptide bond Arg32-Pro33 (loop II). Associated millisecond time-scale motions also affect the conformation and dynamics of the loop I especially in the fragment centered at the Pro7 residue (Lyukmanova et al., 2015). To study the significance of this dynamic pattern, two mutants P7A and P33A were designed. (3) It is assumed that the central loop II of the 'three-finger' toxins is the main structural determinant important for the interaction with their targets (Fruchart-Gaillard et al., 2002; Marquer et al., 2011; Kudryavtsev et al., 2015). To investigate the role of the charged