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ORIGINAL ARTICLE

A Model for Short α-Neurotoxin Bound to Nicotinic Acetylcholine Receptor From *Torpedo californica*

Dmitry Y. Mordvintsev,*[*,1] Yakov L. Polyak,[1] Dmitry A. Kuzmine,[1] Olga V. Levtsova,[2] Yegor V. Tourleigh,[2] and Igor E. Kasheverov[1]

[1]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry RAS, 117997, GSP-7, Moscow, Russia; and [2]M. V. Lomonosov Moscow State University, Biology Faculty, Department of Biophysics, 111992, Moscow, Russia

Introduction

Short- and long-chain α-neurotoxins from snake venoms are potent blockers of nicotinic acetylcholine receptors (nAChRs). Short α-neurotoxins consist of 60–62 amino acid residues and include 4 disulfide bridges, whereas long α-neurotoxins have 66–75 residues and 5 disulfides. The spatial structure of these toxins is built by three loops, I–III "fingers," confined by four disulfide bridges; the fifth disulfide of long-chain α-neurotoxins is situated close to the tip of central loop II. An accurate knowledge of the mode of α-neurotoxin–nAChR interaction is important for rational design of new nAChR agonists and antagonists for medical purposes. Ideas on the topography of toxin–nAChR complexes were based until recently on nAChR interactions with selectively labeled α-neurotoxins, mutations in toxins, nAChR, or both. Recently, crystal structures have been solved for the *Torpedo marmorata* nAChR (4Å [Unwin, 2005]) and for the acetylcholine-binding protein (AChBP) complexed with mollusk α-conotoxin (2.4 Å [Celie et al., 2005]) or α-cobratoxin, long-chain α-neurotoxin (4 Å [Bourne et al., 2005]). However, there were no angstrom-resolution models for complexes of short-chain α-neurotoxins. Here, we report the model of the *Torpedo californica* nAChR extracellular domain complexed to a short-chain α-neurotoxin II (NTII) from *Naja oxiana* cobra venom.

Building of the Models

The basis of this model is a set of ^{1}H-NMR structures of NTII and a wealth of labeling and mutagenesis data available for this and other homologous short-chain α-neurotoxins. When building the model of *T. californica* nAChR extracellular domain, not only the cryoelectron microscopy data for the *T. marmorata* receptor (free of any ligands) were taken into account, but also the data for AChBP complexes with agonists and antagonist. Comparative modeling was done with Modeller program, and toxin-receptor interactions were simulated with the biochemically managed approach under Haddock. The data on labeling with azidobenzoyl derivatives of NTII have been used for this purpose. Subsequent molecular dynamic simulations were compared with data on the mobility and accessibility of spin and fluorescence labels in free and bound NTII. Final model structures were analyzed.

Model Analysis

NTII was located at approx 25–30 Å from the membrane surface. Toxin molecular axis, defined by the direction of the β3-sheet, lies at an ~80° angle relative to the median axis from the center of the nAChR extracellular domain ring and at a small angle to the cylinder wall, practically perpendicular to the membrane surface. About one-third of toxin loop II is

*Author to whom all correspondence and reprint requests should be addressed. E-mail: Chlorine@yandex.ru

plunged into the ligand-binding pocket, located at the interface between two nAChR subunits, whereas loops I and III contact the receptor residues by their tips only. The concave side of loops II and III is very close to the protruding C-loop of the α-subunit, whereas loop I, the amino terminus, and convex side of loop III are solvent accessible. Loop III also contacts the F-loop of the adjacent subunit. The toxin structure undergoes some changes during the final complex formation (for rmsd 1.45 in 15–25 ps), which correlates with earlier NMR data. This position of NTII is in good agreement with the available biochemistry and molecular biology data.

The binding process was shown to be dependent mostly on spontaneous outward movement of the C-loop found earlier in the AChBP complexes with α-cobratoxin and α-conotoxin. Among common features in binding of short- and long-chain α-neurotoxins is the rearrangement of aromatic residues in the binding pocket not observed for α-conotoxin binding. Being in general very similar, binding modes of short- and long-chain α-neurotoxins differ considerably in the manner of loop II entry into nAChR.

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