STRUCTURAL DETERMINANTS OF CARDIOTOXINS MEMBRANE BINDING:
A MOLECULAR MODELING APPROACH

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Key words: membrane-protein interaction, implicit model of membrane, cytotoxin, molecular dynamics

SUMMARY

Motivations: Because of experimental difficulties with characterization of membrane – protein interactions, development of molecular modeling approaches is a field of especial interest. To understand how the membrane binding occurs on molecular level and what structural features are responsible for the strength of binding, we applied a new algorithm, combining molecular dynamics (MD) simulations in water followed by Monte-Carlo (MC) search in implicit membrane, to particular biological objects – two homologous cardiotoxins (CTs), CTI and CTII, from snake venom.

Results: MD simulations of quite structurally rigid molecules of toxins show that CTs differ significantly in structural stability of their loops I and II.

In order to assess the mode of membrane binding for different CTs, their NMR- and MD-derived models were further employed in MC search with implicit membrane. The results obtained reveal the exclusive role of a charged residue in loop II and minor local differences in structure of toxins in their mode of membrane binding.

It is proposed that a long-living water molecule found in the loop II of CT I may play a role in regulating the lipid binding mode of CTs.

INTRODUCTION

A large number of protein molecules are produced by cell either for own cytoplasmic membrane or for interaction with other ones. From the latest – a fair amount of toxins and all of them should have certain adaptive features for efficient overcoming of membrane barrier to damage the cell. One of the interesting toxins' groups is β-structured cardiotoxins from snake venom. CTs belong to the family of the “three-finger” proteins whose fold consists of three β-stranded “finger-shaped” loops protruding from a globular core with four disulfide bridges. Despite the similarity of their spatial models and high level of sequence homology, CTs are characterized by a variety of biological activities and essentially differ in cytotoxicity (Kumar et al., 1997)). It is established now that CTs have unspecific cytolytic effect but little is known about the mode of toxins action on membranes.

Structural and evolutionary comparisons among the CTs family indicated that the major structural plasticity accompanied with the hypervariable amino acid composition is present at the tips of the loops I and II. Also, in a number of three-dimensional (3D) models of CTs a long-living water molecule was found to be tightly hydrogen bonded to the residues of loop II (Sue et al., 2001). This loop has been proposed to be the most important cytolytic domain. CTs have been divided into P- and S-types, depending on the
presence of either Pro31 or Ser29 residues at the tip of loop II. It was found that the P-type CTs interact with bilayers stronger than those of the S-type (Chien et al., 1994; Dubovskii et al., 2005).

Based on NMR data in solution and DPC micelles, spatial structures of both types of CTs, isolated from the same cobra (*Naja oxiana*), CT I (S-type) and CT II (P-type), were established in our laboratory. It was found that they are similar. Moreover, the bound water molecules were identified in their loop II. At the same time, toxins show different degree of cytolytic activity (Feofanov et al., 2004).

It is suggested that the subtle conformational differences in loops extremities as well as possible participation of the bound water may define effectiveness of membrane binding of CTs. Difficulties in getting of such delicate structural information via experiments, make the molecular modeling approaches especially actual. To understand the structural features that may promote the differences in mode of membrane binding, we performed MD and MC simulations of both toxins, respectively in water and in implicit membrane.

**METHODS**

MD in water was used to explore conformational possibilities of CTs as well as to find reliable starting structures for subsequent modeling of CTs binding to membrane using MC conformational search in water-membrane environment.

The spatial models of CT I and CT II determined by NMR spectroscopy in aqueous solution were used as starting structures. Three MD trajectories (~10–22 ns) were obtained for each toxin. In each case stepwise energy minimization and linear heating of the system were preceding the collection run. MD calculations and data processing were performed using the GROMACS v3.1.4 software and a set of original programs. Several MD-conformers as well as experimentally determined 3D models of CTs were used as starting conformations in MC simulations with implicit three-layer membrane model (water–cyclohexane–water). Previously, we have designed a model of the implicit membrane in which the solvent effect was established by the addition of a special term based on the use of empirical atomic solvation parameters incorporated into the potential energy function of a protein in “vacuum” (Efremov et al., 2004). The starting conformations were arbitrarily placed in water, and several successive MC calculations (3–5 x10^5 steps each) were carried out with sampling of 1–2 randomly chosen dihedral angles. Resulting low-energy states in the range of 10 kcal/mol from the minimal energy state were analyzed. Calculations were performed using the FANMEM program (a modified version of the FANTOM package).

**RESULTS**

Analysis of MD data revealed differences in the conformational lability of toxins loop I-II regions. The structure of the loop I of CT II was found to be more stable than that of CT I. On the contrary, dynamic features of CT II may be mainly characterized in terms of structural flexibility of the loop II: some families of MD-conformers differed markedly by conformation of this protein part.

A single water molecule was found to be tightly hydrogen bonded to the following sites of CT I: M26:NH, D29:O (or OD1, OD2), and I32:O. A number of such waters with long residence time (more than nanosecond) were observed in MD simulations. The water molecules bind preferentially in this site, and very rare – in other positions of loop II. To the contrary, multiple binding sites for waters were detected in CT II. Moreover, in a large majority of accumulated MD conformers no bound waters were found in this site.

BGRS'2006
Analysis of MC data shows that the geometry and the depth of CTs’ insertion are determined by the location of hydrophobic loop’s residues (which form apolar surface like a “bottom”) relative to the positively charged conservative residues flanking the ends of loops I-III. Regardless of the starting structures, CT II inserts into bilayer with the hydrophobic extremities of its loops I-III. In case of CT I the mode of binding (via one, two or all three loops) is defined by the conformation of loop II. More precisely, this is determined by the location of the side chain of D29 with respect to the hydrophobic stretch formed by the loop’s residues. In the low energy states this residue is always placed either on the membrane interface or in water.

**DISCUSSION**

Recent studies have shed some light on the structure-activity relationships of CTs. Thus, it has been revealed that the hydrophobic tips of loops I-III represent an important functional motif for binding of CTs to lipid bilayers. Indeed, the results of MC simulations have demonstrated that for efficient penetration into the membrane, the molecule must be able to form a continuous “hydrophobic bottom”. It was proposed that the membrane binding is correlated with the ability of loop II to adopt a $\Omega$-shaped conformation. This promotes formation of a single hydrophobic path (“bottom”) by the loops I-III. Thus, for strong binding with membrane the loop II of CTs must contain mainly apolar residues. Also, it should have some features constraining its conformational mobility to favor the “right” conformation of this loop on the membrane interface. Indeed, the loop II of CT II is quite hydrophobic and has residue P30 (like other representatives of the P-type CTs) at its tip. Finding the bound waters among several P-type toxins suggests that this proline residue should play an important role in formation of the water binding $\Omega$-shaped loop II. For the P-type toxin, CT II, effectiveness of its membrane binding was confirmed by a series of MC searches starting from several distinct conformational states founded in MD.

As shown from MC data analysis, the presence of charged residue D29 in the loop II of CT I substantially confines a number of states that may realize the hydrophobic stretch. As a consequence, the mode of membrane binding via one or two loops appeared among the low-energy states of CT I.

Recent NMR studies of CTI revealed the presence of a bound water molecule located near the tip of loop II and its absence in aqueous and micellar environment, respectively. The results of MD simulations of CT1 are completely consistent with the experimentally derived information. Moreover, the water binding site identified with the two independent techniques is identical.

It seems that the absence of Pro30 can make this loop too flexible. But the additional hydrogen bonds holding water molecules near the residues M26, D29, and I32 make the structure of loop II more rigid, thus avoiding significant conformational changes. Indeed, as seen from MD data, the loop II of CT I was less mobile as compared with that of CT II, where bound water molecules were observed much rarely. Comparison of the two NMR-derived 3D models of CT I, in solution and in membrane-like environment (DPC micelles), reveals only one substantial difference between the structures – namely, the conformation of the tip of loop II and, as consequence, opposite orientations of side chains of D29. Note, that the NMR-structure in DPC micelle does not contain a water binding site. The geometry of binding of such NMR-structure was similar to that of CT II: all three loops interact with the bilayer.

MC simulations with implicit membrane suggest the role of this particular conformation of loop II. Probably, it provides favorable orientation of the charged group of D29: away from the “hydrophobic bottom” formed by the extremities of loops I-III. We hypothesize that preserving of the definite loop II conformation through the binding
of water molecule favors its conformational “switching” into the “right” conformation (accompanied with the release of water) on the water-membrane interface.

ACKNOWLEDGEMENTS

This work was supported by the Russian Foundation for Basic Research (grants 04-04-48875-a, 05-04-49283-a) and by the Russian Federation Federal Agency for Science and Innovations (The State contract 02.467.11.3003 of 20.04.2005, grant SS-4728.2006.4).

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