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ORIGINAL PAPER

The role of glycine residues at the C-terminal peptide segment in antinociceptive activity: a molecular dynamics simulation

Yong-Shan Zhao · Rong Zhang · Yang Xu · Yong Cui · Yan-Feng Liu · Yong-Bo Song · Hong-Xing Zhang · Jing-Hai Zhang

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Abstract Elucidating structural determinants in the functional regions of toxins can provide useful knowledge for designing novel analgesic peptides. Glycine residues at the C-terminal region of the neurotoxin BmK AGP-SYPU2 from the scorpion *Buthus martensii* Karsch (BmK) have been shown to be crucial to its analgesic activity. However, there has been no research on the structure–function relationship between the C-terminal segment of this toxin and its analgesic activity. To address this issue, we performed three MD simulations: one on the native structure and the other two on mutants of that structure. Results of these calculations suggest that the existence of glycine residues at the C-terminal segment stabilizes the protruding topology of the NC domain, which is considered an important determinant of the analgesic activity of BmK AGP-SYPU2.

Keywords Bmk · Molecular simulation · Molecular dynamics · Toxin

Introduction

Scorpion α-toxins are single-chain polypeptides consisting of 60–72 amino acids [1–4]. These α-toxins can be divided into three subfamilies according to their activities against mammals and insects: classical α-toxins, α-like toxins, and insect α-toxins [5, 6]. Classical α-toxins or anti-mammalian toxins, such as Aah2 or Lqh2, are highly toxic to mammals and weakly active towards insects [7, 8]. Insect α-toxins, such as LqhαIT, have been found to be especially active towards insects when tested by intracerebroventricular (icv) injection [9–11]. The α-like toxins, such as BmK-M1, act on both insects and mammals [12–14].

Most scorpion α-toxins share a common framework consisting of three double-stranded antiparallel β-sheets linked to a short α-helix, despite their pharmacological diversity (Fig. 1) [15–17]. Extensive mutagenesis and three-dimensional structural elucidation suggest that the putative functional surface of a scorpion α-toxin can be divided into two domains [18–20]. The NC domain consists of a five-residue turn (residues 8–12) and a C-terminal segment (residues 56–64). Residues in the loops preceding the α-helix and between the β2 and β3 sheets form the core domain. It has been shown that the core domain is involved in the binding preference of the receptor. However, the NC domain is considered to determine the toxin specificity, due to its unique tertiary arrangement and the fact that changes in the amino acid sequence have been observed. A flat topology of the NC domain is suggested to play a role in the anti-mammalian toxin, while a protruding conformation seems crucial to high insecticidal potency (Fig. 2) [9, 21, 22].

BmK AGP-SYPU2 had been isolated and purified from the venom of the *B. martensii* Karsch and shown to have analgesic activity at the animal level [23]. A sequence determination showed that the mature peptide is composed of 66 amino acid residues and considered a classical α-toxin according to sequence homology and phylogenetic analysis (Fig. 3) [24]. Our previous studies showed that glycines 6566 of BmK AGP-SYPU2 are involved in its analgesic activity, and that substitution of these two residues disturbs its biological properties [25]. In this study, we investigate the structure–function relationship of BmK AGP-SYPU2

Y.-S. Zhao · R. Zhang · Y. Xu · Y. Cui · Y.-F. Liu · Y.-B. Song · J.-H. Zhang (✉)
School of Life Science and Bio-pharmaceutics, Shenyang Pharmaceutical University, Shenyang, Liaoning Province 110016, People's Republic of China
e-mail: jinghaizhang82@gmail.com

H.-X. Zhang
State key Laboratory of Theoretical and Computational Chemistry, Institute of Theoretical Chemistry, Jilin University, Changchun 130023, People's Republic of China

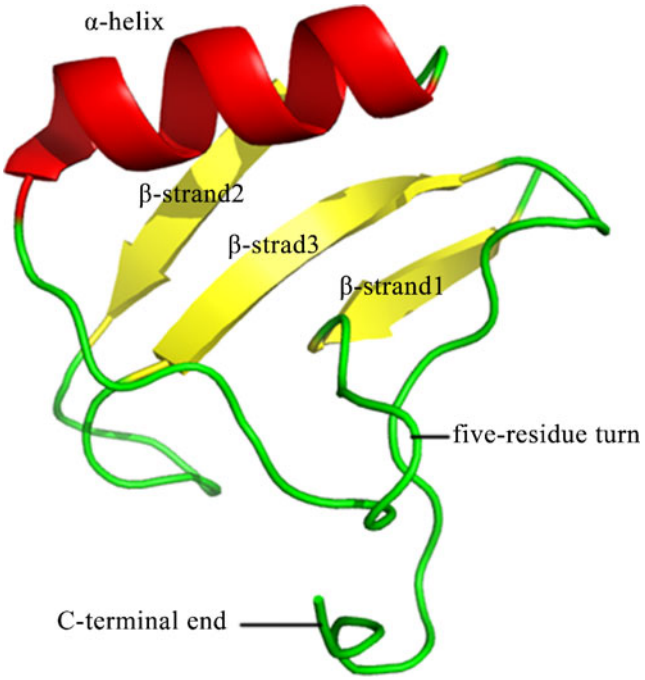

Fig. 1 The common fold of α-toxins. The α-helix is shown in *red*, the β-strand in *yellow*, and the loops in *green*

using molecular dynamics simulations. Based on the results obtained, we suggest that glycines at the C-terminal segment maintain the NC domain's protruding topology, which is associated with high analgesic activity.

Materials and methods

Atomic coordinates and model construction

The initial coordinates of BmK AGP-SYPU2 were retrieved from the Protein Data Bank (PDB ID: 2KBH) [24]. The same PDB file with reduced was used to construct two mutants. The first mutant model was obtained by deleting one glycine at the C-terminal end (Mut66) and the second by deleting two glycines at the C-terminal end (Mut6566) using the PyMOL software package [26].

Molecular dynamics

All simulations were performed using the Amber12 software package together with the ff99SB parameters for proteins, and the Ptraj module of Amber12 was used to analyze the computational results [27, 28]. The starting models were solvated in a rectangular box of TIP3P (explicit water model) water molecules with a minimum distance of 12 Å between any protein atom and the box boundaries. To neutralize the models, three chloride ions were added [29].

Prior to MD simulation, a series of minimizations were performed. All water molecules were first minimized while restraining the positions of the atoms of the protein with a harmonic potential. The whole system was then energy minimized without restraint for 2,000 steps using a combination of the steepest descent and conjugated gradient

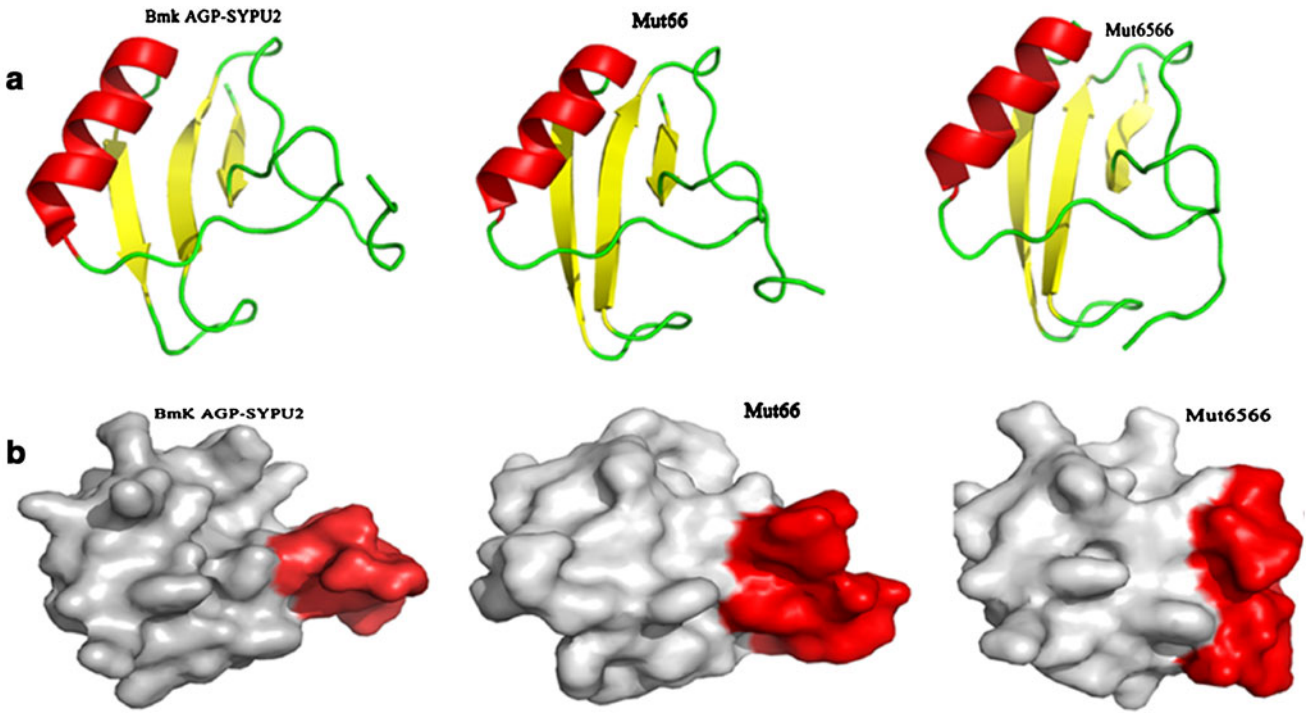

Fig. 2 **a** 3D structures of the wild type (WT) of BmK AGP-SYPU2 and mutations of the WT. **b** Disposition of the NC domain (in *red*) in the WT and its mutations. Note that this domain protrudes in the WT and is flat in Mut6566 toxin

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                          *         20          *         40          *         60
BmK AGP-SYPU2: VKDGYIADDRNCPYFCGRNAYCDGECKKNRAESGYCCWASKYGNACWCYKLPDDARIMKPGRCNGG
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Fig. 3 Amino acid sequence of BmK AGP-SYPU2

methods. After gradually heating the system from 10 to 300 K over 100 ps using the NVT ensemble, a 1 ns simulation was performed at 1 atm and 300 K with the NPT ensemble to equilibrate the whole system. For production runs, MD simulations were performed in the NPT ensemble for 100 ns.

For all simulations, all bonds involving hydrogen atoms were constrained using the SHAKE algorithm [30]. A time step of 2 fs and a non-bonded interaction cut-off radius of 10 Å were used. The particle-mesh Ewald (PME) method was employed to calculate long-range electrostatic interactions [31]. During the sampling process, the coordinates were saved every 5 ps for further analysis.

Results and discussion

Global and local structural behavior

To the best of our knowledge, MD simulation is a suitable tool for investigating the effect of mutations on protein structure and dynamics [32–36]. In this work, conventional MD simulations of three systems—BmK AGP-SYPU2 (the wild type, WT), Mut66, and Mut6566—were carried out in an explicit water model (TIP3P) for 100 ns. To gauge the dynamic stabilities of the three simulated systems, the root mean square deviations (RMSDs) of the heavy atoms from their starting coordinates were calculated and plotted in Fig. 4. The RMSD values for the WT and mutated systems remained stable and followed the same trend, indicating that the global structures of the three systems are quite similar to that of the initial system. To clarify their local structural features, the root mean square fluctuation (RMSF) per residue was monitored. As shown in Fig. 5, the largest RMSFs were seen for residues in the C-terminal segment. In particular, the overall fluctuations of residues 56–62 increased when glycines 6566 were deleted. Our previous studies showed that site-directed mutations of the C-terminal region could affect the analgesic activity of BmK AGP-SYPU2. In particular, the activity of Mut6566 was significantly decreased compared to that of BmK AGP-SYPU2. This indicated that there is a strong correlation between the analgesic activity and the C-terminal segment.

Toxin shape and size

To further study the effect of mutation on the structure of BmK AGP-SYPU2, the average structures of the three systems during the last 5 ns of simulation were calculated. As shown in Fig. 6, the spatial arrangements of the three systems are similar except for the orientation of the C-terminal segment, in agreement with the discussion above. To quantify the effects of mutation on the stability of the C-terminal segment, the distance between the alpha carbon of His64 at the C-terminal end and that of Lys41 in the loop connecting the β2 and β3 strands was measured. As shown in Fig. 7, this distance is generally smaller for the mutants than the WT, and the distance for Mut6566 is the smallest, which indicates that the C-terminal segment of Mut6566 shows the largest deviations from the starting structure.

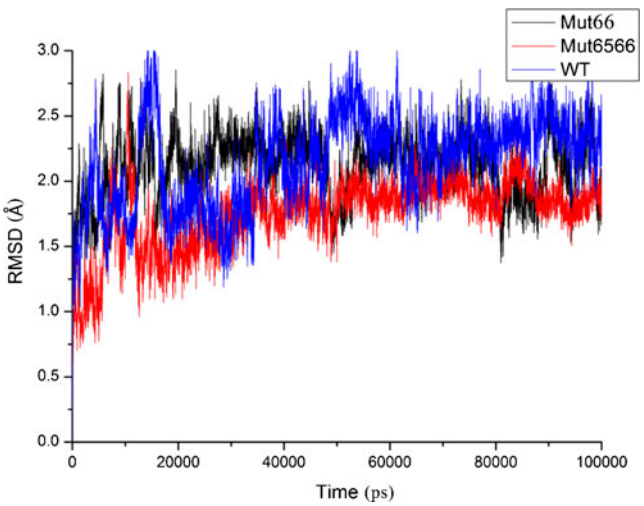

Fig. 4 RMSD values of the WT and two mutations of the WT—Mut66 and Mut6566—as a function of simulation time (in ps)

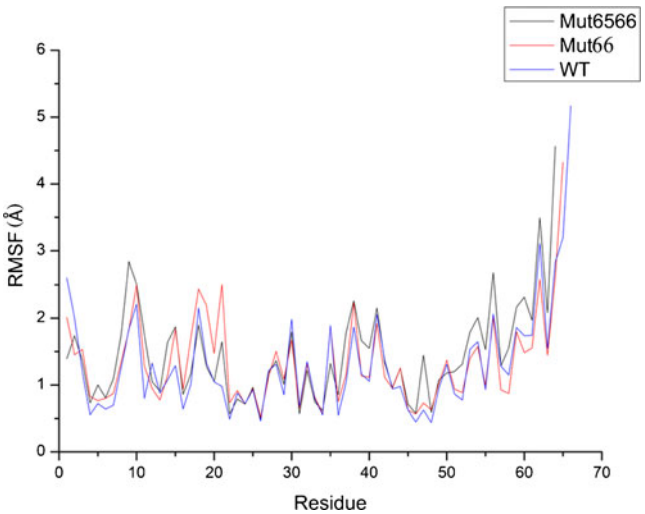

Fig. 5 Changes in the positions of alpha carbons in the WT, Mut66, and Mut6566 compared with their positions in the native state

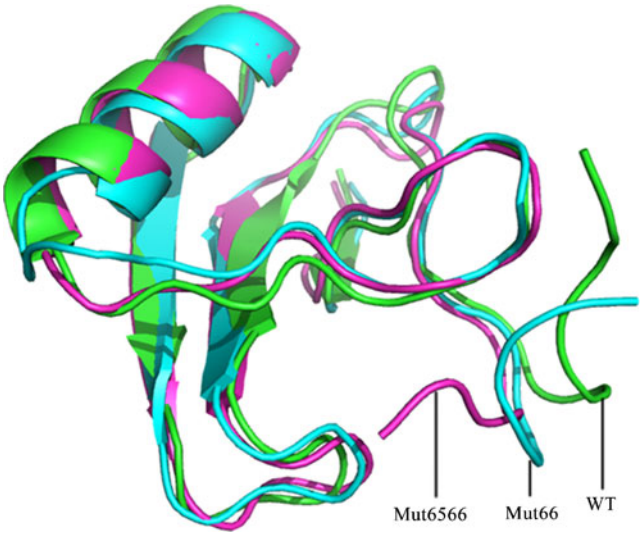

Fig. 6 Comparison of the refined WT model with its mutations. The *green ribbon* is a representation of the WT. The *blue ribbon* is a representation of Mut66 and the *pink ribbon* is a representation of Mut6566

Moreover, it is clear that the C-terminal end of Mut6566 is far from the five-residue turn and close to the loop connecting the β2 and β3 strands, and the NC domain has a flat topology in the absence of the Gly6566 residues. By contrast, the NC domain has a protruding topology in the WT, whereas the topology of the NC domain in Mut66 is intermediate between the NC domain topologies seen for Mut6566 and the WT. Further analysis of the structure of BmK AGP-SYPU2 suggests that the hydrophobic core of the NC domain in Mut6566 is significantly disrupted, resulting in the flat topology of its NC domain, which leads to significantly decreased analgesic potency of the protein. Therefore, the conformation of the NC domain of the WT is probably influential in the interaction of the WT with the analgesic receptor, due to the protruding topology of its NC domain.

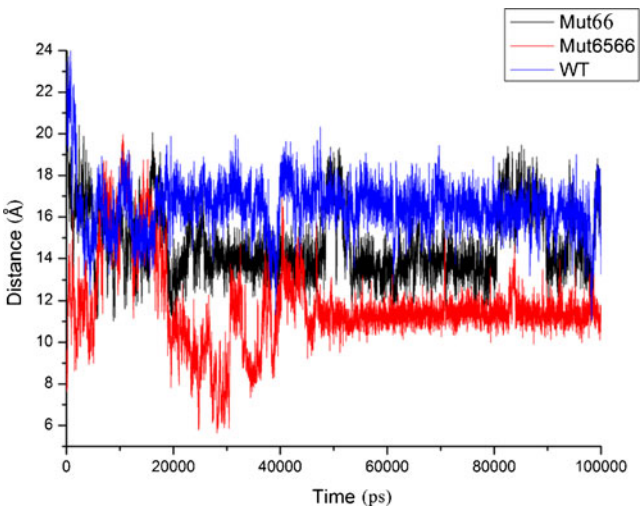

Fig. 7 Distance between the centers of mass of residues His64 and 46 in the WT, Mut6566, and Mut66

Recently, two models of α-toxins complexed with the VS domain of $Na_v1.2$ have been proposed by Wang et al. and Chen and Chung [37, 38]. Both of these models suggest that the NC domain is involved in receptor site binding, even though the complexes predicted by the models are different. In the present study, our models show that the existence of glycine residues at the C-terminal segment stabilizes the protruding topology of the NC domain. When the NC domain has a protruding topology, it is conceivable that some key amino acid residues in it are well positioned to interact with the receptor site, and are thus important for the analgesic activity of BmK AGP-SYPU2.

Conclusions

In the present study, we performed long-range MD simulations of BmK AGP-SYPU2 and two mutant forms of it in order to investigate the structural differences among these molecules at the atomic level. The overall protein topologies of the WT and the mutated systems were similar except for the C-terminal segment. Mut6566 shows a significantly different topology of the NC domain compared to that seen in the WT, and exhibits no analgesic activity. We hope that the work described in this paper provides a possible structural explanation for the change in analgesic activity that occurs when the topology of the NC domain is modified from flat to protruding, and that this information proves useful in the design of new specific analgesic peptides.

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