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Adjusting force distributions in functional site of scorpion toxin BMK M1 by cooperative effect of disulfide bonds

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Abstract

We decided to investigate the influence of the presence of disulfide bonds on the pattern of force distribution in the functional site of scorpion toxin BMK M1 in its functional state. Therefore, a series of short time molecular dynamics (MD) simulations were performed on this toxin in the native state and disulfide bond broken states. The comparison of disulfide bond broken states with the native state showed that the electrostatic potential energy of important functional residues in the reverse turn and C-terminal regions were modulated by the cooperative effect of all disulfide bonds in the molecule. Furthermore, our results revealed that disulfide bonds also play a cooperative role in modulating (1) the amplitude of the fluctuations of the functional segments and (2) the correlation of motions between important functional residue pairs in this toxin. Therefore, we can conclude that the disulfide bonds have cooperation to adjust the pattern of force distribution in the functional site of this toxin in its functional state.

Keywords: Adjusting force distribution; Cooperative effect; Disulfide bonds; Functional state; BMK M1

Disulfide bonds are the only common covalent cross-links in proteins. Experimental and theoretical studies so far have revealed the contribution of these covalent bonds to folding, conformational stability, and catalytic activity in proteins. Disulfide bonds as well as non-covalent interactions overcome the loss of conformational entropy associated with folding that destabilizes native conformation [1]. On the other hand, according to the two current models, these cross-links enhance stability mainly through denatured state effects [2–4]. Since disulfide bonds seem to play different roles in different states, one may ask "*what is the role of disulfide bonds in the functional state of proteins?*" There is some experimental evidence that some disulfide bonds are more important for tuning function than for structural stability and folding [5]. An attractive example is the disulfide bonds in scorpion toxins; it has been reported that in the scorpion toxin scaffold, modifications of conserved and interior cysteine residues could permit modulation of function, without significantly affecting folding efficiency and structure [6]. In addition, disulfide bonds seem to influence the patterns of correlated and concerted motions, which are expected to have a key role as modulators of the functionally important motions [7,8].

Beside these covalent bonds, electrostatic interactions as long-range forces control important aspects of structure and function in proteins. These forces play a crucial role in protein interactions with ligands or other proteins. Since these forces guide binding process, the calculation of electrostatic potentials and the investigation of the factors that influence these forces is a prolific field of research. Electrostatics, in particular, operates at large distances and can enhance or impede bimolecular collision rates [9–11].

Since the pattern of internal motions, correlation of motions, and electrostatic force distributions in proteins seem to play an important role in molecular recognition and function, we investigated the influence of the presence of disulfide bonds on these patterns in the functional state of scorpion toxin BMK M1. In experimental studies, this effect was usually submerged by their more rugged role in

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folding and stability. A short timescale of MD simulations does not allow significant structural deviations from the native state. Therefore, we can use short time MD simulations to investigate this aspect of disulfide bond efficacy without being influenced by large-scale structural changes.

Representative scorpion toxin BMK M1 with 64 residue cross-linked by four disulfide bonds from the scorpion *Buthus martensii karsch* is an good model for this study (Fig. 1). Since scorpion toxins are able to adopt stable and biologically active structures, constitute a priori interesting candidates as core structure for protein engineering and design [12]. These neurotoxins are known to interact specifically with the voltage-dependent sodium channel and affect sodium conductance in various excitable tissues, and thus serve as important pharmacological tools for the study of excitability and sodium channel structure.

BMK M1 belongs to α-like toxins targeting sodium channel and it has been reported that electrostatic interactions play a crucial role in the function of this toxin [13]. This miniprotein is composed of a dense core of secondary structure elements, including an α-helix and a three-stranded antiparallel β-sheet. Three β-turns connect these secondary structures. There are also four loops including Lys8-Cys12, Trp38-Asn44, Arg58-His64 residues and a long loop spanning Val13-Arg18 between the first loop and α-helix. In fact, the segment Lys8-Cys12 is a reverse turn that together with C-terminal loop Arg58-His64 forms functional site in this toxin, called site RC. Three disulfide bridges formed by residues Cys16-Cys36, Cys22-Cys46, and Cys26-Cys48 stabilize the special βαββ motif in this toxin. The fourth disulfide bond formed by Cys12-Cys63 stabilizes the loop Arg58-His64 in site RC [14]. Elimination of the disulfide bonds by site-directed mutagenesis in this molecule showed that Cys22-Cys46 and Cys26- Cys48 disulfide bonds are essential for the general folding, Cys16-Cys36 bond is a crucial structural element for stabilizing the general fold, and the Cys12-Cys63 bond is essential for the toxic activity and the binding property with the Na^+ channel [15].

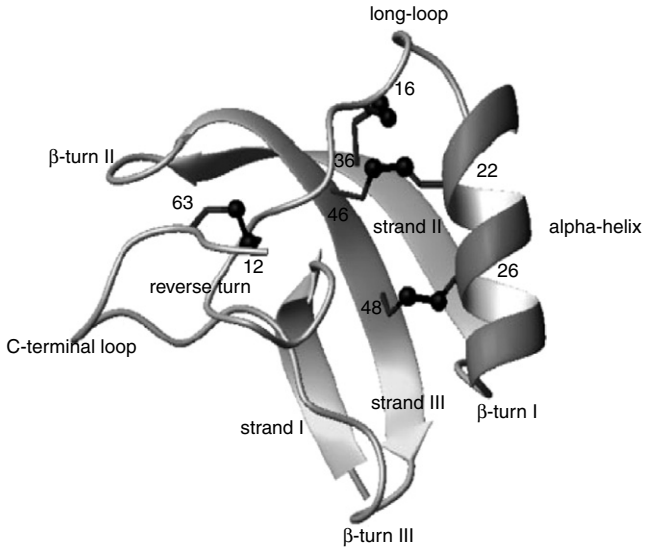

Fig. 1. Structure of scorpion toxin BMK M1.

Results of toxicity tests and binding studies have revealed that site RC is a functional site in this toxin. The C-terminal basic residues Arg58, Lys62, and His64 together with Lys8 in the reverse turn are critical for bioactivity of the molecule [13]. So the pattern of force distribution in this site should be important for function in this toxin.

In this study, we performed a series of short MD simulation times (2-ns) on BMK M1 in the native state, individual disulfide bond broken states, and also all disulfide bonds broken state. We investigated the influence of the presence of disulfide bonds on the patterns of internal motions, correlation of motions, and electrostatic force distributions in the functional site of this toxin.

Methods

Computational procedure. The initial coordinate of BMK M1 was obtained from the Protein Data Bank (PDB) with entry code 1SN1 [14]. The same conformations with the reduced form of cysteines were used as the initial structure of the disulfide bond broken states in MD simulations. For all simulations, the 6 Na^+ and 8 Cl^- charge-balancing counterions were added in order to neutralize charges on the protein surface.

Both initial setup and dynamics runs were carried out by Amber 8 [16]. All calculations were performed with a cutoff distance of 10 Å. The protein was solvated with TIP3P model of water [17] in an octahedron box with a minimum 8 Å distance between the box edge and the closest portion of the molecule. First, the system was energy minimized for 500 steps of steepest descent minimization to remove close van der Waals contacts and to allow formation of hydrogen bonds between water molecules in the periodic box and the protein. Temperature was increased from 200 to 300 K during a 100-ps MD simulation in the canonical ensemble (NVT). A 100-ps MD simulation in the isobaric–isothermal ensemble (NPT) was carried out to equilibrate the system in constant pressure. The density was stabilized around 1.02 g/cm^3 during equilibration phase in constant pressure. Temperature and pressure were controlled by applying a weak coupling method [18], with temperature and pressure relaxation times $\tau_T = 1.0$-ps and $\tau_p = 1.0$-ps, respectively.

MD simulations were performed in the NPT ensemble for 2-ns during production phase. Time step of 2-fs was used for all simulations and X–H bonds were constrained using the SHAKE algorithm [19]. Translational center of the mass motions was removed every1000 steps and coordinates were saved every 0.4-ps for analysis.

Analysis procedure. The Ptraj module of AMBER was used to extract Root-mean-square deviations (RMSD) and root-mean-square fluctuations (RMSF) data from trajectories.

One of the most frequently used measures to assess the stability of a MD simulation over the course of time is the RMSD between experimental coordinate and the generated structures in the trajectory, as follows:

$$\mathrm{RMSD} = \sqrt{\frac{1}{N}\sum (r_i^{\mathrm{exp.}} - r_i^{\mathrm{gen.}})^2} \qquad (1)$$

where $r_i^{\mathrm{exp.}}$ and $r_i^{\mathrm{gen.}}$ denote the Cartesian coordinates for atom i in the experimental and generated structures, respectively.

The dynamical properties of C_α atoms have been reported to contain sufficient information to investigate the most important motions in proteins [20]. Therefore, RMSF of C_α atoms was used to investigate structural flexibility. Prior to calculating the RMSF, we removed the overall translational and rotational motions by superimposing backbone of each snapshot structure onto the one in the starting structure of the trajectory, using the least-squares fitting method.