Abstract

Two kinds of scorpions are living in Yeyama islands, Japan. One of them is Yaeyama scorpion (*Liocheles Australasiae*). Interestingly, the venom of *Liocheles Australasiae* displays the toxicity for insects but almost not for mammals. LaIT1, 36 residues peptide, is the most major toxic peptide in the venom from *Liocheles Australasiae*. And detail of the structure and function of LaIT1 had been reported. Unlike LaIT1, little is known about the second major component, a 59 residues peptide termed LaIT2, forming three intramolecular disulfide bridges. The biological study had shown that the N- and C-domains of LaIT2 possess antimicrobial and insecticidal activities, respectively. Although LaIT2 had been predicted to take the β-KTx fold (a helix plus an α-β-β motif), the three-dimensional structure is still unknown. Despite the intriguing biological activities of LaIT2, lacking the three-dimensional structure prevents to uncover the feature of functional mechanism at the molecular level. To clear this issue, in this thesis, I solved the NMR structure and analyzed the molecular dynamics of LaIT2. Moreover, activity measurements of LaIT2 and the mutants were performed to find the key residues for the functions.

Firstly, the sample preparation method was established. To induce the formation of intramolecular disulfide bonds and to enhance the solubility of the target peptide, LaIT2, the pET32a vector and *E. coli* named Rosetta-gami B (DE3) pLysS were employed. Combination of Ni^{2+}-affinity column, enzymatic digestion, and HPLC enabled to obtain the recombinant LaIT2 (rLaIT2) at sufficient level of purity. Mass spectrometric analyses of the rLaIT2 fragments yielded by Lys-C, Asp-N, and/or proteinase K digestions showed that the rLaIT2 formed intramolecular disulfide bonds identical to those of natural LaIT2; Cys^{31}-Cys^{51}, Cys^{38}-Cys^{56}, and Cys^{42}-Cys^{58}. In addition, insecticidal and antimicrobial activity test showed that the activity of rLaIT2 is almost identical to that of natural LaIT2.

Next, the solution structure and molecular dynamics of LaIT2 were investigated using NMR spectroscopy. A series of homo- and hetero-nuclear two- and three-dimensional NMR experiments were performed. All NMR spectra were recorded at 298K on a Bruker AVANCE III NMR spectrometer equipped with a triple-resonance TCI-cryogenic probe at the ^{1}H resonance frequency of 800.13 MHz. After the resonance assignments, distance and angle constraints were collected. To satisfy these structural constraints, the three-dimensional structure of LaIT2 was calculated using CYANA2.1. The final set of 20 structures showed that LaIT2 has a β-KTx-like structure; the N-terminal domain is in random coil conformation and the C-terminal domain forms the cysteine-stabilized αβ (CSαβ) fold. Moreover, NMR and CD data of LaIT2 in the presence of Trifluoroethanol (TFE) and liposomes showed that the unstructured N-domain has an ability to form an α-helix. The results strongly suggested that LaIT2 can form the canonical β-KTx fold in certain condition. The conformational plasticity of the N-domain would contribute to the target recognition mechanism. Furthermore, the results of T_1, T_2, and $^{15}N\{^{1}H\}$-NOE experiments suggested that A24 and Q28 in the N-domain have large thermal and conformational flexibility, respectively. The flexibility of these two residues is thought to prefer to adopt an appropriate angle between the two domains for the target recognition.

Finally, important amino acid residues for the biological activities were deduced based on the structural comparison of LaIT2 with four homologous peptide toxins suggested by BLAST search. The comparison showed that K15 and K21 in the N-domain and L53 and L54 in the C-domain were well conserved. Therefore, three mutants, K15A, K21A, and L53A/L54A were prepared to examine their insecticidal and antimicrobial activities. The activity measurements suggested that K21 in the N-domain is necessary for both activities. Moreover, in the results, K15 in the N-domain was found to be responsible for the antimicrobial activities, whereas L53 and L54 in the C-domain were key residues for the insecticidal activity. The findings in this thesis will provide a new insight to understand the structure-function relationships of LaIT2 and β-KTx family members.

Keywords: NMR structure, Scorpion venom, Antimicrobial activity, α-β-β motif, *E. coli*