Structural Studies of Fragments from Sticholysin II

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Abstract: *Sticholysin II (St II) is a citolytic protein produced by the anemone Sthichodactyla helianthus, which interacts with membranes disrupting their permeability barrier. In this work, we have studied the structure and dynamics of three fragments from St II that were identified as the region that could adopt an amphipatic α-helix conformation, and thus could be responsible for the interaction of the protein with the membrane. Structural analysis has been carried out by circular dichroism (CD) and ^{1}H-Nuclear Magnetic Resonance (^{1}H-NMR). CD results showed that the peptide VLDKVLEELGKVSRKIAVGI-NH_2, St II (16-35), in sodium dodecyl sulfate (SDS) micelles acquires the maximum helical content. Thus, we investigated the structure of St II (16-35) by ^{1}H-NMR in SDS micelles. The results show that the peptide is indeed predominantly helical, with a regular amphipathic α-helix, spanning the region from Leu^{17} to Ala^{32}. The surface of the bacterial membrane is anionic and the helical surface includes positively charged amino acids residues that could be the key for the interaction with the bacterial membranes. These structural features, together with the fact that the peptide does not present hemolytic activity, point to St II (16-35) as a promising candidate for the design of new antimicrobial substances.*

Sticholysin II (St II) is a cytolytic protein produced by the Caribbean Sea anemone *Sthichodactyla helianthus*. This toxin interacts with biological and model membranes destroying their permeability barrier through a mechanism related to pore formation.[1] Thus structural studies of St II are important not only to understand its toxic properties, but also to investigate basic molecular mechanisms of processes such as polypeptide insertion into membranes, pore assembly, and solute permeation through the pores. In addition, St II has potential biotechnological and pharmaceutical applications.[2]

The lytic mechanism of St II has not been clarified yet. An important region that could be involved in toxin-membrane interaction is an amphipathic α-helix located at the N-terminus. It carries a cluster of positively charged residues that could interact with membrane acidic lipids. Such interaction has been demonstrated for Equinotoxin II, which reveals

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a 64-66% sequence identity to St II.[1] In this work we have studied the structure of three fragments from St II (Table 1) with the aim of understanding the molecular mechanisms underlying St II-membrane interaction. These protein fragments were synthesized, as they encompass regions predicted to adopt an amphipathic α-helix conformation, and could therefore be potentially involved in toxin-membrane interaction. The structural analysis has been carried out by circular dichroism (CD) and ^{1}H Nuclear Magnetic Resonance (^{1}H-NMR).

Table 1. Sequences of fragments from St II.

St II (1-30)	ALAGTIIAGASLTFQVLDKVLEELGKVSRK-NH_2
St II (11-30)	SLTFQVLDKVLEELGKVSRK-NH_2
St II (16-35)	VLDKVLEELGKVSRKIAVGI-NH_2

CD experiments were performed on Jobin Yvon CD6 and Jasco J-810 spectropolarimeters. 1D and 2D homonuclear NMR experiments were recorded at 20 °C on a Varian Inova 500AS spectrometer operating at 11.7 T. NMR data were processed using the nmrPIPE/nmrVIEW software. Structure calculations were performed using the Dyana software. The resulting structures were energy minimized by the DISCOVER module of INSIGHT II package (Accelrys Inc.).

The CD spectra of the three peptides in water showed that they are in a random coil conformation. For St II (1-30), the increase of pH, ionic strength, and peptide concentration induce the formation of a coiled coil structure. In the case of peptide St II (11-30), instead, a coil-coiled structure is promoted only upon increase of ionic strength, while changes in peptide concentration and pH do not produce any effect. Fragment St II (16-35) maintains a random conformation upon variation of pH, ionic strength, and peptide concentration.

As a model for protein-lipid interaction, the peptides were studied in the presence of SDS micelles. In this condition, St II (16-35) turns out to be the peptide exhibiting the higher helicity as compared to the other two. Interestingly, this fragment does not present hemolytic activity, and when it is incubated with erythrocytes, inhibits the hemolytic activity of St II. Based on these structural features and recognizing that the peptide/protein biological activity involves membrane interaction, the 3D structure of St II (16-35) was determined in SDS micelles by ^{1}H NMR spectroscopy. An NMR sample was prepared by dissolving St II (16-35) to the final concentration of 1 mM in 600 μL of a 100 mM SDS-d_{25} solution, 5% of D_2O and pH ~ 4. As expected, the NMR-derived structure of St II (16-35) in SDS micelles (Figure 1) is predominantly helical, with a regular α-helix spanning the region from Leu^{17} to Ala^{32}.

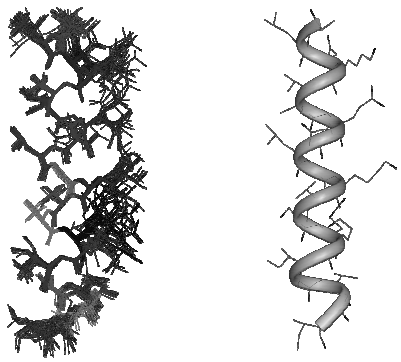

Figure 1. 40 final structures (left) and ribbon representation of the lowest energy structure (right) of St II (16-35).

The structure reveals that the α-helix is amphipathic, with the hydrophobic face involving residues Leu^{17}, Val^{20}, Leu^{21}, Leu^{24}, Val^{27}, Ile^{31} and the hydrophilic side presenting the residues Asp^{18}, Lys^{19}, Glu^{22}, Glu^{23}, Lys^{26}, Arg^{29}, Lys^{30}. The helical surface includes four positively charged amino acids residues (Lys^{19}, Lys^{26}, Arg^{29} and Lys^{30}) and they could be the key for selective interaction with the bacterial membranes.

These structural features together with the fact that the peptide does not present hemolytic activity, point to St II (16-35) as a promising candidate for the design of new antimicrobial compounds.

In addition, the results support the hypothesis that, upon interacting with the erythrocyte membrane, the peptide competes with St II for binding, leading to the inhibition of the toxin's hemolytic activity.

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References

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