NMR structure of the sea anemone cytolisin Sticholysin I

I. Castrillo, J. Santoro, M. Bruix

Dept. of Spectroscopy and Molecular Structure, IQFR, CSIC,
Serrano 119, 2006 Madrid, Spain

Sticholysin I (StnI) is an actinoporin, a pore forming toxin, produced by the sea anemone *Stichodactyla heliantus*. Together with Stn II it is the most potent cytolisin produced by this anemone. These proteins have the singularity of being produced as water soluble forms that are able to interact with membranes. Upon biding to the membrane they change their conformation, produce oligomeric pores in the membranes and cause cell lisation [1].StnI shares 94% of sequence identity with Stn II, despite this high similarity, they have been reported to have different lipid dependence and different haemolytic activity, both being higher for Stn II than for Stn I [2]. These two proteins are a very good model systems for the study of conformational changes involved in the transition from the water-soluble state to the membrane-bound one, as well as for investigating protein-protein and protein-lipid interactions that take place during the pore formation.

Here, Stn I soluble structure has been determined by NMR spectroscopy. Doubly labelled ^{13}C ^{15}N Stn I was produced using an *E. coli* expression system. NMR spectra were recorded on a Bruker AV-800 spectrometer at 25ºC. Samples were prepared both in 90% H_2O/ 10% D_2O and in D_2O at pH 4.0. Nearly complete assignment of ^{1}H, ^{13}C and ^{15}N resonances was achieved using the following spectra: ^{1}H-^{15}N-HSQC, ^{1}H-^{13}C-HSQC, HN(CO)CA, HNCA, CBCA(CO)NH, CBCANH, HNCO, HC(C)H-TOCSY, (H)CCH-TOCSY, HACANH and HBHACONH. Inter proton distances were derived from 3D ^{15}N- and ^{13}C-NOESY in 90%H_2O/ 10% D_2O and 2D NOESY spectra in D_2O. Angle restraints were obtained using the programs TALOS and PREDITOR. Distance restraints and structure calculation were carried out in a semiautomated iterative manner using the CYANA 2.1 software package.

The Stn I structure consists of a β-barrel sandwich composed by 10 β-strands, flanked by two short α-helices on each side. The structure is in general well defined. The regions with higher RMSD values correspond to the loops between β4-β5 and β6-β7 strands. Dynamic studies, based on heteronuclear relaxation, are in progress to understand the possible intrinsic flexibility of these regions.

The StI structure will be compared with other actinoprines of known structures from the same family, like Stn II or EqtII. These studies would contribute to our understanding of the molecular processes directing pore formation.

[1] Parker, M. W.; Feil, S. C. *Prog Biophys Mol Biol.* 2005, *88*, 91-142.
[2] Valcarcel, C. A.; Dalla Serra, M.; Potrich, C.; Bernhart, I.; Tejuca, M.; Martinez, D.; Pazos, F.; Lanio, M. E.; Menestrina, G. *Biophys J.* **2001**, *80*, 2761-74.