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Structure of the hemolytic toxin FraE helps to clarify the evolution of actinoporins.

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We have expanded the structural catalogue of actinoporins, a family of highly potent hemolytic proteins belonging to the class of pore-forming proteins. Herein we report the crystal structure of a new actinoporin that we termed FraE at 2.2 Å resolution. This structure improves our understanding of the evolution of this family of toxins.

1 Introduction

Actinoporins are pore-forming proteins produced by sea anemones known for their widespread polymorphism [1]. Actinoporins have conserved functional sites to ensure the preservation of their mechanism of pore-formation, a complex process involving several different steps [2]. Among them, the oligomerization of ~8 units is driven by protein-protein interactions that are conducive to the assembly of the functional pore in target membranes [3].

Previous studies identified variability in residues at the protein-protein interaction surface. Herein we have isolated a new variant of actinoporins termed FraE. By using X-ray crystallography, we aim to understand if the protein-protein surface and other key regions are conserved in the family of actinoporins.

2 Experimental

FraE was cloned into the pBAT4 vector. Expression of FraE was carried out in *Escherichia coli* BL21(DE3). Expression was induced with 0.5 mM IPTG for 20 hours at 20 °C. Cells were harvested, lysed with a probe sonicator, and the supernatant subjected to centrifugation. The protein was subsequently purified by ion-exchange and size-exclusion chromatography.

Crystals of FraE were obtained in a solution composed of 0.2 M sodium formate, 20% w/v PEG 3350 at pH 7.2. Crystals were transferred to a solution of mother liquor supplemented with 20 % (v/v) glycerol and fast-frozen in liquid nitrogen. Diffraction data was collected at BL-5A of the Photon Factory (Tsukuba, Japan) under cryogenic conditions (100 K). The crystal structure of FraE was determined by the method of molecular replacement using the homolog protein FraC (PDB code 3VWI). The crystal was determined at 2.2 Å resolution and deposited in the Protein Data Bank under entry code 6K2G.

3 Results and Discussion

A new actinoporin termed FraE was identified and cloned. We The crystal structure of FraE was determined at a resolution of 2.2 Å. FraE exhibited similar structure and hemolytic properties to FraC and Eqt-II, two well studied actinoporins (Fig. 1) [2,4]. Based on these crystal structures and their primary sequences, we identified regions of high structural similarity such as the lipid binding site and the protein-protein interaction surface, and other regions of significantly less similarity (Fig. 2).

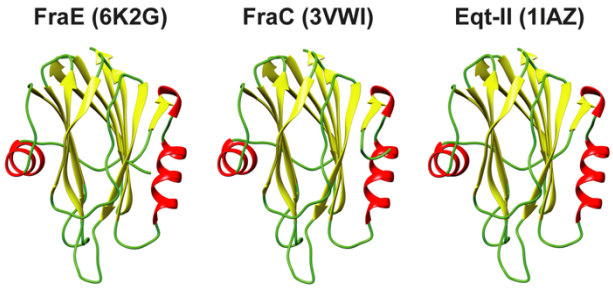

Fig. 1: Structure of three actinoporins. Root-mean square deviation of their coordinates was less than 0.5 Å.

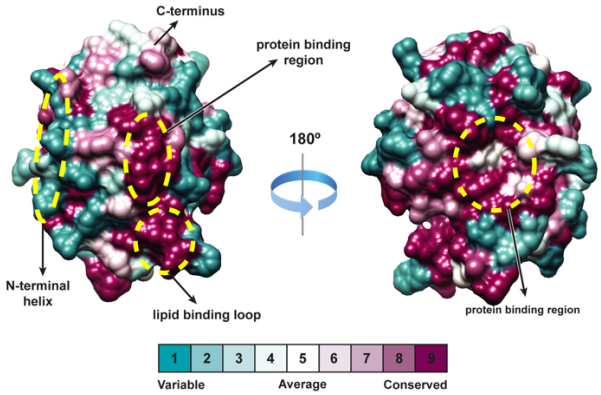

Fig. 2: Evolutionary variability of actinoporins.

We conclude that actinoporins are a homogeneous group of proteins with very similar architecture, but also with local differences that may explain their target selectivity.

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