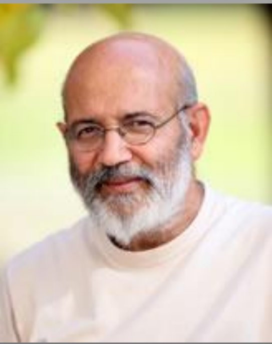

THE ROLE OF ENDOPLASMIC RETICULUM OXIDOREDUCTIN1 IN THE FOLDING OF CONOTOXIN

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Cone snails (*Conus*) are a genus comprising approximately 700 species of venomous marine mollusks. Each snail contains a variety of conotoxins most of which are specific to that species. Conotoxins are small peptide neurotoxins that are highly specific ligands for ion channels and receptors of the nervous system. Most conotoxins contain disulfide bonds, which are critical for their biological activity.

Protein disulfide isomerase (PDI) is an enzyme responsible for the formation of disulfide bonds during protein folding in the endoplasmic reticulum of eukaryotes. As PDI introduces disulfides it becomes reduced and must be re-oxidized to continue the formation of disulfides. Another enzyme, endoplasmic reticulum oxidoreductin1 (Ero1), is involved in the process of re-oxidizing PDI allowing for the continuation of disulfide formation. Ero1 makes use of the cofactor flavin adenine dinucleotide (FAD), which allows it to donate electrons directly to molecular oxygen.

This study aims to monitor the ability of Ero1 isolated from *Conus geographus*, a species of cone snail, to aid PDI in the formation of the correct disulfides using conotoxins as substrate. cDNA was synthesized from RNA isolated from the venom gland of *Conus geographus*. DNA encoding Ero1 was amplified by PCR using specific primers designed based on previous transcriptome data. The amplified DNA was cloned in *E. coli* and its sequence determined. Sequence analysis revealed that the DNA did encode Ero1. The encoded protein was 432aa long and contained 16 cysteine residues. It was then aligned with 100 other species, including vertebrates and invertebrates, to determine conserved cysteine residues. Vertebrates are known to have two isoforms of the Ero1 enzyme that differ slightly in cysteine residues. Ero1 from the cone snail was compared to both isoforms to determine if it was more closely related to either of the vertebrate isoforms. The alignment showed that some key regions, such as the FAD binding domain, were conserved but the enzyme as a whole was not. When compared with the two vertebrate isoforms, cone snail Ero1 contained the essential cysteine residues of both.

After sequence analysis, Ero1 was expressed in *E. coli* using the pGEX-6P-2 vector. The vector expresses the Ero1 with a glutathione S-transferase tag, creating a GST-Ero1 fusion protein. The GST-Ero1 fusion protein was purified by affinity chromatography with glutathione beads. PreScission Protease was then used to cleave the GST from Ero1. The Ero1 was further purified by repeating the affinity chromatography to remove GST, but also by size exclusion chromatography to remove Ero1 dimer.

A folding assay with Ero1 and PDI from *Conus geographus* was performed by adding synthetic linear conopeptides to a mixture of the two enzymes, and following the time course of the reaction after quenching the reaction with formic acid. Using HPLC the samples were examined for the amount of properly folded toxin. The folding assay revealed that there was more properly folded toxin when Ero1 was present in addition to the PDI. This project determined that cone snail Ero1 is an active enzyme and that it interacts with PDI to more efficiently fold conotoxin.