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Microcrystal electron diffraction of the peptide Gramicidin D

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Microcrystal electron diffraction (micro-ED) is a cryo-TEM technique that can be used to determine the atomic structure of proteins, peptides, and small molecules [1]. Micro-ED data is obtained by continuously tilting the crystal in the cryo-TEM while recording diffraction information [2]. This method has been successfully used for the structure determination of proteins such as proteinase K, lysozyme, adenosine A2A receptor (G-protein coupled receptor) and peptides such as an Alzheimer associated amyloid-β (20-34), SVQIVY (tau protein fragment) and others [3-7].

Growing diffraction quality crystals of proteins and peptides can be challenging. X-ray diffraction techniques typically require crystals that are at least 1 μm in each direction. Crystals smaller than that could not be utilized in single crystal diffraction studies until the advent of micro-ED. In this technique the ideal thickness of the crystal perpendicular to the incident beam is in the nanometer range [2].

In this contribution we report on our research to determine the structure of gramicidin D, a peptide antibiotic produced non-ribosomally by Bacillus brevis [8]. It acts, in part, by creating pores in membranes, rendering them incapable of supporting life-sustaining transmembranal gradients. Gramicidin is a highly apolar pentadecapeptide consisting of alternating D- and L-amino acids. Naturally occurring gramicidin is a mixture of isoforms: gA (80%), gB (6%), and gC (14%). The amino acid sequence of gA is:

Formyl–NH–L–Val–Gly–L–Ala–D–Leu–L–Ala–D–Val–L–Val–D–Val–L–Trp–D–Leu–L–Trp11–D–Leu–L–Trp–D–Leu–L–Trp–CO–NH–CH2–CH2–OH.

In gB and gC, Trp at position 11 is replaced by L-Phe and L-Tyr, respectively. The ion conducting form of gD is generally considered to be a dimer. Gramicidin exists in two major conformations; a head-to-head, single stranded helical dimer and a left- or right-handed intertwined, parallel or antiparallel, double stranded double helix [9].

The peptide was dissolved in a mixture of ethanol / PEG 4000 and crystallized in batch-mode at 4°C. Small plate-like crystals formed. The crystals in the presence of ethanol / PEG 4000 mixture were transferred onto Quantifoil R 2/2 grids and access solvent was removed by vacuum suction. The grids were flash frozen in liquid nitrogen and transferred into a Glacios cryo-TEM equipped with a Ceta-D camera. Datasets from several crystals were successfully collected. We will discuss the data processing in XDS and our progress on solving and refining the structure.

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