Poster Presentation

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Structural characterization of aminoglycoside modifying enzyme ANT(2")-Ia

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Aminoglycosides are a class of broad-spectrum antibiotics used in the treatment of serious Gram-negative bacterial infections, they target the 16S RNA subunit and upon binding cause errors in translation, eventually inducing a bactericidal effect [1]. Aminoglycoside nucleotidyltransferase (2")-Ia (ANT(2")-Ia) is an aminoglycoside modifying enzyme that prevents aminoglycosides from binding to the ribosomal subunit, making this enzyme a principle candidate structure-based drug design [2]. Characterization of ANT(2")-Ia has been proven to be difficult due to the low stability and solubility of overexpressed protein, where 95% of the protein being expressed is in the form of inclusion bodies [3]. We describe a protocol that has lead to successful expression and purification of ANT(2")-Ia. A successful enzymatic assay has also been adapted and the protein is active and stable under these conditions with a specific activity of 0.14 U/mg. Furthermore, nuclear magnetic resonance (NMR) studies have allowed for the assignment of 144 of the 176 non-proline backbone residues. Substrate binding NMR experiments have shown unique global chemical shift perturbations upon binding ATP and tobramycin, suggesting unique binding sites for each substrate. Structural determination of ANT(2")-Ia using NMR in conjunction with x-ray crystallography can be utilized in order to develop small molecules that will act as more effective aminoglycosides in order to inhibit ANT(2")-Ia from binding and modifying these antibiotics.

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