TITLE: Characterization of a Zinc Finger Protein with a High Affinity for the REV Binding Site of the Human Immunodeficiency Virus

AUTHOR: Beatrice Edjah

FACULTY SPONSOR: Dr. Markus Germann, Professor, Chemistry Department

Keywords: Zinc Finger, HIV, Human Immunodeficiency Virus, NMR

Introduction:

Zinc fingers (ZnF) are small protein modules that are coordinated by a zinc ion with various combinations of cysteines and histidines. In this study, a designed ZnF29G29R was purified and characterized due to its high affinity to the RREIIB, a substructure in HIV RNA to which REV binds to. Interference of the REV binding caused by the ZnF29G29R inhibits the export of viral RNA and is therefore of therapeutic interest. NMR spectroscopy will be employed to study the interactions between the ZnF and the HIV RNA.

Methods:

Two sets of ZnF29G29R, one grown in full media and the other in minimal media, were expressed as fusion proteins, ZnF+Thioredoxin (THX)+His_6-tag, in an *E.coli* culture. Using a nickel affinity column, the fusion proteins were extracted from the lysate and dialyzed. The ZnFs were cleaved from the Thx+His_6-tag using the enzyme enterokinase and were separated using a cation ion exchange column. Size exclusion allowed for further purification of the proteins.

Results:

Using a UV-Visible spectrophotometer, it was determined that a 2L full media expression resulted in 17 mg of protein and a 6L minimal media expression produced 16 mg of protein. Purity of the proteins was accessed using SDS-PAGE gels and proper folding of the proteins was determined by observing the Phe14 and His27 δ2H chemical shifts using ^{1}H NMR.

Discussion:

With the use of various chromatography columns, the ZnFs were properly purified. Using the same methods, isotopically (^{13}C, ^{15}N) labeled ZnF will be used to bind to the HIV RREIIB RNA sand the interactions will be properly studied using NMR.