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Study of the Interaction between TET Protein and Vitamin C

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Ten-Eleven Translocation (TET) enzymes are essential components of biological processes due to their role in DNA methylation. According to the Article, "Structure and Function of TET Enzymes", found on Pubmed.gov, TET enzymes have been found to oxidize the carbon-C5 position of cytosine (5mc), where methylation of DNA primarily occurs, to various derivatives. (Pubmed) This project focuses on this enzyme, because its mutations have been found to occur in myeloid disorders, such as Acute Myeloid Leukemia. The other component of the project is ascorbic acid, which is commonly referred to as Vitamin C. The article, "Vitamin C Induces TET-Dependent DNA Demethylation and a Blastocyst-like State in ES Cells", published by *Nature* suggests that ascorbic acid enhances TET activity by inducing demethylation of DNA in only the TET components. (Nature) The goal of this project was to explore this interaction experimentally.

A NOESY 2D NMR experiment was conducted to view the interaction between the TET protein and ascorbic acid. NOESY 2D NMR is very useful for determining protein structures, as explained by Horst Schirra on the website, Two-Dimensional NMR Spectroscopy. NOESY uses the dipolar interaction of spins to correlate protons that are close in the amino acid sequence, as well as in structure. (Schirra) Although there are three TET enzymes, the original goal of the project was to study TET2 protein and ascorbic acid. Studies have shown that TET mutations found in Acute Myeloid Leukemia patients are directly linked to the TET2 enzyme. However, purchasing TET2 protein to conduct the experiment was unsuitable for the project budget. Therefore, to conduct the 2D NMR experiment, TET1 and TET3 were used. Using deuterated water, pure samples of TET1 and TET3 were produced, as well as both TET enzymes with ascorbic acid. A total of four samples were prepared. Along with using 2D NMR to view the interaction between the TET protein and ascorbic acid, a protein-ligand molecular model was produced using the online docking server, SwissDock. Protein Data Bank files of TET1 and TET3, and the molecular structure of ascorbic acid were submitted to the docking server, which generated predictive models of the two enzymes and ascorbic acid. The models provide a visual idea of what the interaction, indicated by the 2D NMR results, would look like.

Based on the results of the 2D NMR experiment and the presumptive docking models, the interaction can be explored. Interpretation of the spectra, and an understanding of ascorbic acid's structure provide information regarding where the ascorbic acid binds to the TET protein, as well as the amino acids that are involved. Literature provided by *UniProt Consortium* suggests that the binding positions of both enzyme's respective amino acid chains involve Tyrosine, Glutamine, and Histidine. (UniProt) Further research can be conducted, mirroring this project, to explore the interaction of TET2 and ascorbic acid. Thus, leading to drug design research for Acute Myeloid Leukemia.

Citations

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