Briefly Describe your preparation and interest in performing undergraduate research with your chosen mentor

My preparation for performing undergraduate research includes not only BI118, BI218, CH118, and CH218 (The integrated Science Experience I and II classes), but also BI271 in which I worked in collaboration with Dr. Tolan. Together in BI271, we dissected scientific papers regarding the topics of nonalcoholic fatty liver disease (NAFLD) and the various pathways that are being studied in order to learn the cause and (hopefully) find the cure.

My interest in the accumulation of fatty liver disease stems from my family. As a child of one parent who has lost over forty pounds in one year, I have been curious as to the causes behind obesity. Throughout this time and as of current, my mother has changed her eating habits to include hardly any processed foods; she mainly consumes vegetables, fruits, lean proteins, and whole grains. In the time that my mother has lost this weight, her entire personality seems to have changed in addition to her health. I am very interested not only in biological pathways that can affect the human body in such a large way, but also how exactly consuming processed foods affect our bodies negatively.

Dr. Tolan's research lab will allow me to explore one of the most important pathways in the accumulation of fatty liver disease. This is not only exciting for me because it is something of interest and of personal experience, but I know I will learn and greatly benefit from working with Dr. Tolan and my other colleagues at the lab.

Research Title: Targeting ketohexokinase as a therapy for hereditary fructose intolerance and metabolic syndrome.

Describe the research problem (100-500 words)

Over the past 40 years there has been n steady increase in metabolic syndrome diseases such as diabetes and obesity that highly correlate with an increase in the consumption of added sugars in beverages and foods. The primary sugar responsible for the rise is these diseases is D-fructofuranose or fructose, which is a 5-membered carbon ring found in many plants. Ketohexokinase (KHK) is a key enzyme in fructose metabolism that converts fructose into fructose 1-phosphate (Figure 1). There are two isoforms of KHK in humans that differ in exon three due to alternative splicing (Figure 2). KHK-C has a higher affinity for fructose and is found in the liver, kidneys, and intestines[1]. A majority of dietary fructose metabolism occurs in the liver, therefore future experiments will be focused on the C isoform. In addition to diabetes and obesity, there are inborn errors in metabolism such as Hereditary Fructose Intolerance (HFI) that disrupt fructose metabolism. HFI results from a deficiency in aldolase B, which causes a buildup of fructose 1-phosphate in the blood and can be fatal especially in infants[2]. The proposed project seeks to investigate various inhibitors of KHK-C and their binding sites to this enzyme through x-ray crystallography. By inhibiting KHK-C, excess fructose would be excreted in the urine and there would be no accumulation of fructose 1-phosphate, alleviating both fructose induced obesity and HFI. Working towards this goal, upwards of 400 botanical extracts and 1200 phytochemicals were screened for their inhibitory activity of KHK[3]. With the help from collaborators, multiple promising candidates for inhibition of KHK-C will be surveyed and analyzed through the use of x-ray crystallography. The hypothesis is are there unique binding sites for inhibitors of ketohexokinase?

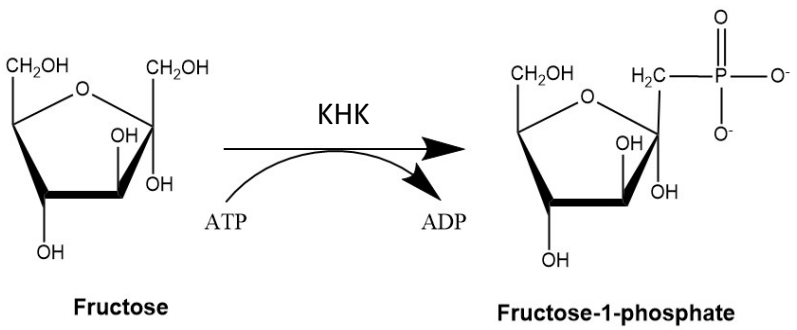

Figure 1. Initiation of Fructose Metabolism: Ketohexokinase binds fructose in an ATP dependent reaction to form fructose 1-phosphate.

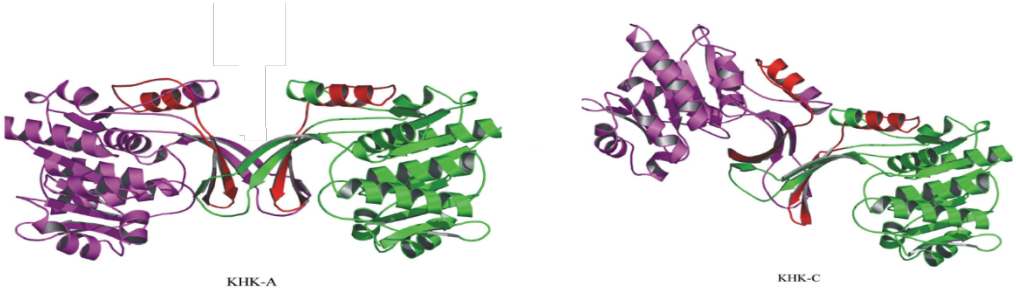

Figure 2. Isoforms of Ketohexokinase: Isozymes of KHK, labeled accordingly, in similar conformations showing alternative splicing results in red. The red section contains residues 71-115 (Trinh 2009).

What methods will be used? (100-500 words)

Currently mouse KHK-C (mKHK-C) is being used to determine binding sites of inhibitors while the construct for human KHK-C (hKHK-C) is being cloned into the high expression vector pPB1. This is being done through PCR and other biochemical techniques. The hKHK-C sequence is currently in the low expression vector pPal8, which was obtained by our collaborators at UCD. We plan on using PCR to amplify the gene out of pPal8, then transfer it into pPB1. Once the new clone is created, we will express and purify the protein, then proceed to crystallography studies.

How will the data be collected and analyzed? (100-500 words)

In collaboration with the Allen Lab at Boston University, multiple crystal screening trays will be set up and any crystals formed will be exposed to high energy x-rays, yielding a diffraction pattern that can be used to solve the structure. Hanging drop vapor diffusion will be done in 24-well plates to co-crystalize KHK with various inhibitors to identify optimal crystallization conditions. These crystals will then be harvested and sent to high energy x-ray sources such as the Stanford Synchrotron Radiation Lightsource where diffraction data will be collected. The data will then be analyzed by computational modeling programs such as Phenix[4] and COOT[5] which will perform molecular replacement and refinement on models created from the data. These models of KHK-C bound to a small molecule inhibitor will give insight into the effectiveness of the inhibitors, as well as elucidate their mechanism of action, with the hope of using the inhibitors as alternative treatments for HFI and metabolic syndrome.

Cited Sources

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