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Determining Amyloidogenicity of Islet Amyloid Polypeptide (IAPP) in Animal Species

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Abstract:
A main cause of Type-II Diabetes has been identified as the clumping and aggregation of certain proteins within the body. Misfolding causes these insoluble, fibrous proteins, known as amyloid proteins,, thus causing fibrous protein deposits within the body that lead to toxic effects. Islet amyloid polypeptide (IAPP) is the polypeptide known to result in protein misfolding and aggregation in the pancreas, but is present in both healthy and diabetes-affected individuals. IAPP, released by pancreatic B-cells, is secreted with insulin to maintain healthy glucose levels within the body, but cell conditions can cause IAPP to have amyloidogenic properties, thus killing such B-cells. It is known that rats and mice cannot contract type-II diabetes, but humans, cats, and monkeys can. It is also known that these animals that cannot contract diabetes also do not have aggregating IAPP, whereas the organisms that can contract the disease have these aggregating proteins. This experiment will aim at discovering and targeting the regions in which mutation occurs in IAPP and developing ways in which we can diminish the aggregation of IAPP.

Introduction: Type-II Diabetes: The Oligomer Hypothesis

With 1.7 million American people being diagnosed each year, diabetes stands as a huge threat to the American population (American Diabetes Association). Specifically, type-II diabetes, which represents 90-95% of Americans with diabetes, has proliferated immensely within the last ten years, but has been closely connected to the lifestyle choices that individuals make, such as eating or exercise, as well as through one's genetic predisposition.

Biologically, diabetes is caused by the aggregation of toxic proteins that build up fibers within the pancreas. Aggregation is defined as the clumping of proteins within the body. Although there are many theories established regarding the cause of diabetes, most scientists have come to accept the toxic oligomer hypothesis. The toxic oligomer hypothesis states that protein hormones within the pancreas can produce toxic oligomers, a molecular complex that is made up of a few small units, that kill pancreatic cells (Haataja et. al, 2009). Peptides are the basic unit of proteins in the human body. A regulatory peptide, known as Islet Amyloid Polypeptide (IAPP), becomes toxic by misfolding, and this malfunction leads to cell death within the pancreas. These cells, known as beta cells, make up parts of the pancreas and secrete insulin and IAPP, in order to regulate the amount of glucose, or energy, available to the body. When IAPP aggregates within the body, it kills B-cells, and the pancreas decreases the release of insulin, causing high, unregulated glucose levels (Moffet, 2011). Diabetes is directly related to this issue: when glucose is too high, and the body is not producing enough insulin to lower it back to homeostatic levels, diabetes emerges.

For nearly three decades it has been known that mouse and rat IAPP do not aggregate and that these two organisms do not develop type 2 diabetes; however, the IAPP of monkeys, humans, and cats do aggregate and these organisms do develop type 2 diabetes. While about six

or eight other organisms have been studied as well, there have been no comprehensive studies to try to connect more data points in this fashion. In a partnership with the San Diego Zoo, we will begin a comprehensive study of animal IAPP aggregation in an effort to explore microbiological differences in DNA sequencing for the protein IAPP. We will continue our study of these sequences by testing compounds that have the possibility of decreasing IAPP clumping in the pancreas.

Materials and Methods

The San Diego Zoo has sent us the genetic material from several organisms not known to develop diabetes such as elephant, rhino, camel and hippo. We will clone the IAPP gene from each of these organisms and have it sequenced. Simultaneously, we will generate a collection of animal IAPP-EGFP sequences to study for their ability to allow EGFP, a protein connected to the gene, to fluoresce. If the compound fluoresces, this is evidence that there is clumping involved, and that the IAPP protein is becoming toxic to the cells.

We have begun making transformations of genetic material by uptaking the animal sequences into plasmids. Plasmids are segments of circular DNA that can easily uptake genetic material when put under specific conditions. We will transfer the DNA segment of the animal IAPP and insert it into a plasmid to test for clumping of the IAPP protein. We will use the compounds from Dr. McCallum's lab to test whether they have the ability to decrease the clumping of IAPP, a mechanism that could potentially halt the progression of type-II diabetes.

Background: What do scientists know about Islet Amyloid Polypeptide and Type-II Diabetes?

Studies show that over 40 diseases are caused by a protein or peptide misfolding or structurally malfunctioning, causing the protein or peptide to lose its proper function. In most

cases, these malfunctioning proteins result in amyloid deposits, which include plaques and fibers that build up extracellularly, causing toxins in the body (Hudson et al. 2009). Connections have been drawn between the presence of IAPP in the body and the satiety regulation, or the feeling of being full. In most diabetes patients, health, specifically one's diet, plays a major role in the onset of diabetes, but strong connections can be drawn between IAPP and ones genetic makeup. Although IAPP is mostly found in the B-cells of the pancreas, there are traces within the stomach that could have possible effects on the eating habits of individuals (Hataaja et al. 2008).

Several different researchers have explored the possibility for drugs and compounds to stabilize aggregation of IAPP so that it does not form amyloid proteins, reduce the concentration of amyloid in the body, inhibit amyloid production, and remove toxic oligomers and fibers once they are already formed. Amyloid is simply defined as a sticky protein that clumps together in the cell and creates plaque that can be toxic to its surrounding environment (Westermark et al. 2011). Although some drugs have been created that have managed the symptoms and effects of amyloid aggregation, a drug has yet to exist that will diminish the aggregation and presence of amyloid, and prevent it from occurring within our bodies. This amyloid derives from normal proteins that we all have in the body, but in some conditions they misfold, and in response create insoluble amyloid aggregations that our bodies are unable to break down (Borman, 2010).
All humans contain IAPP, but roughly 95% of type-2 diabetes patients contain IAPP with toxic amyloid properties in their pancreas. Studies have concluded that amino acid region 20-29 on IAPP can be referred to as the amyloidogenic region, thus affecting amyloid formation and contributing to the toxic plaque build-up (Fox et al, 2009). My mentor, Dr. Moffet, has researched possible mechanisms for reducing or inhibiting IAPP using compounds that can target the amino acid region 20-29. Polyphenols have been studied as plausible inhibitors of IAPP

aggregation. Dr. McCallum's chemistry lab has synthesized similar compounds that we will test on the IAPP samples, in hopes of finding a compound to inhibit the aggregation of these proteins. If the aggregation can be inhibited, there is a possibility that we will have the basis for a possible mechanism for treatment of type-II diabetes.

Summer Research Expenses

My research expenses for the summer would be covered by the NIH grant that Dr. Moffet has received to conduct our research. This grant, however, does not include living arrangements, which I would have to arrange for the entirety of the summer. The table below illustrates my rent breakdown for the apartment I would be living in.

Table 1: Summer Budget

Expenses	Cost
Rent (May-August)	$1170 per month for 4 months
Total	$4680

This research grant led my research group on a great path last summer, and we have been successful and made immense progress in our field. I hope to continue this research this upcoming summer in order to continue improving our work and creating publishable research. I am extremely thankful for the opportunities that the Honors Program has provided me with thus far, and I hope to continue to disseminate my work throughout the remainder of the semester.

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