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The Effects of Cation Compounds on Amyloid Proteins in Type-II Diabetes

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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. Cations such as Na+, Zn+, Cu2+, have shown potential properties that can reduce or slow IAPP aggregation within the cell. Thioflavin T is a dye that increases fluorescence when it binds with an amyloid, thus acting as a good indicator of amyloidogenic properties within IAPP samples. IAPP will be analyzed through a fluorescence spectrophotometer, which excites electrons in molecules through ultraviolet light, causing the molecules and electrons to emit visible light. These emittances can be viewed through graphical format and analyzed to determine how much IAPP aggregated, and how much the cation compounds inhibited aggregation, if at all.

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.

In my exploration of this phenomenon, I would like to examine the mechanisms for reducing or slowing the rate of IAPP aggregation within the body, in hopes of providing a remedy

for type-II diabetes. I will specifically focus on compounds that will reduce the aggregation of IAPP, thus decreasing the amount of toxic oligomers and B-cell death within the pancreas.

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. These mechanisms include injecting the human amyloid protein with metal compounds that can inhibit clumping, and allowing beta cells to perform their normal function of secreting insulin. These metal (cation) compounds found directly in the lab of the Loyola Marymount Biochemistry Department have shown evidence to increase the clumping of amyloid proteins, and my research will focus on the ability for metal compounds to hopefully inhibit aggregation and provide a possible treatment for diabetes.

Methods: How will I introduce cation compounds to IAPP and analyze the rate of aggregation?

In this experiment I will utilize samples of IAPP, that have been pre-made and purchased, and test them with various cations to see if any cation compounds have the ability to reduce IAPP aggregation. An initial sample of 375μL of IAPP will be speed vacuumed to evaporate any liquid from the sample, leaving only the dried amyloid polypeptides. Three samples, a control and two experimental tubes, will be prepared with IAPP and TRIS buffer. TRIS buffer is a common buffer used in biology and biochemistry because it holds a pH between 7.8-9, making it a good buffer that imitates elements of the human body. The two experimental tubes of IAPP will contain 135μL of

TRIS buffer with 15 μL of a specified cation compound (CuCl2, ZnCl2, etc). The control tube will contain 150μL of TRIS buffer, but no cation compound. Utilizing the control, I will compare the IAPP aggregation with that of the IAPP plus cation sample to compare their differing levels of aggregation. I will run each sample through a Fluorescent Spectrophotometer. An aliquot will be prepared containing 17μL of the sample and 663μL of a fluorescent dye, Thioflavin-T. Thioflavin-T binds to IAPP and shows fluorescence that corresponds with the amount of amyloid fibrils present. One at a time, each cuvette will be placed into the Fluorescent Spectrophotometer to analyze its fluorescence and aggregation of IAPP. If the IAPP shows curves that rise above 700 nm, it has shown aggregation and can be considered dangerous to the body. In contrast, I am hoping that the IAPP and cation samples will have curves much lower than the control, showing that the metal compounds had an effect on inhibiting the aggregation of amyloid proteins. Each set of samples (control and two cation containing tubes) is tested every 5 minutes, starting at ten minutes and ending at forty, to analyze the ability for IAPP to aggregate over time.

To further my research, I will examine the IAPP samples using Atomic Force Microscopy (AMF). This method is utilized to detect the fibrous components that exist when the sample is placed onto a slide. The imaging of AMF will show a microscopic view of the aggregation of proteins, and the various heights of the proteins will allow us to determine the various stages of amyloid formation over time. With this method, I can analyze the slides with the various cation compounds and compare which compounds were able to inhibit, if at all, this build-up of aggregate.

Expected results: What effect will cation compounds have on the aggregation of IAPP?

The Fluorescent Spectrophotometers show curves that symbolize the aggregation of the IAPP samples. In this experiment, it is hoped that IAPP control samples will show curves of over 700nm, but that cation plus IAPP samples will inhibit or slow aggregation. If curves from cation and IAPP samples are considerably lower on the graph in comparison to the control, this means that cation compounds were able to inhibit or slow aggregation. Throughout the forty minutes, IAPP generally aggregates more over time, but I am hopeful that the cation compounds can resist this increase in aggregation, and actually fight against it to inhibit the aggregation of amyloids.

Thus far, copper has been tested and has shown likelihood of inhibition of IAPP. The fluorescent peak stayed under 100 nm, which showed significant evidence for preventing the clumping of the amyloid protein. Within the lab, we will continue to test copper in order to get a sufficient number of trials, and ultimately produce a scientific paper on our findings. Our next focus will be on cobalt and zinc cation chlorides, and these trials will continue next semester. One final aspect of the research we are performing is making sure to find out what compounds are harmful to the body. If we avoid this, the findings we come up with could be toxic to the human body and unable to be utilized in the biomedical field.

Conclusion

The research and treatment provided towards type-II diabetes costs the American public $174 billion a year (American Diabetes Association). The amount of people in America with the disease is 9.3%, but is rising by roughly 2% each year since 2010. With the research of Dr. Moffet and myself, I believe that it is possible for us to make immense progress on a possible cure for type-II diabetes. With simple metal compounds, the amyloid proteins in our bodies can be

regulated, and the human population will have a mechanism for fighting against this debilitating disease.

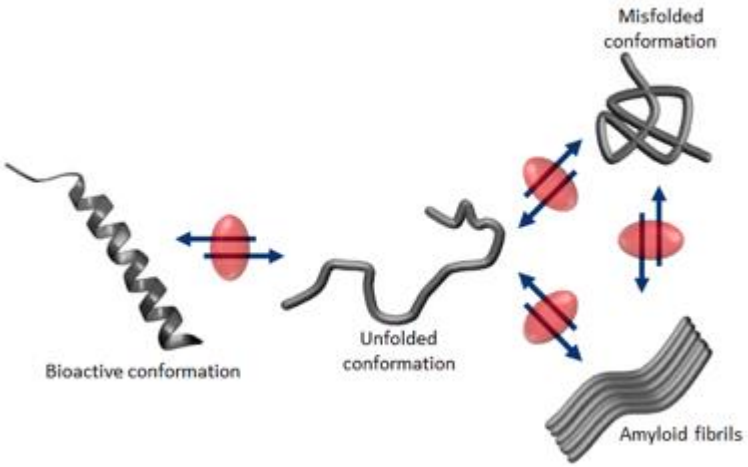

Figure 1: Image of amyloid proteins clumping due to misfolding.

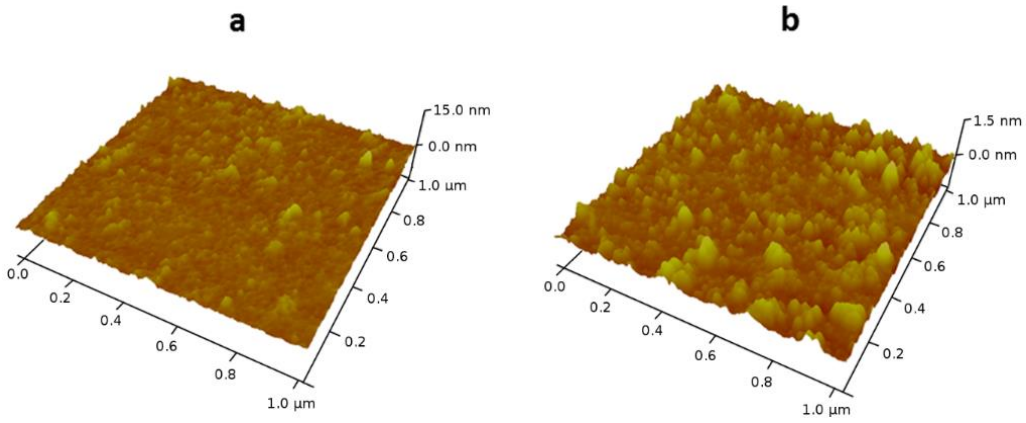

Figure 2: Fluorescent Spectrophotomer used for looking at aggregation of amyloid proteins

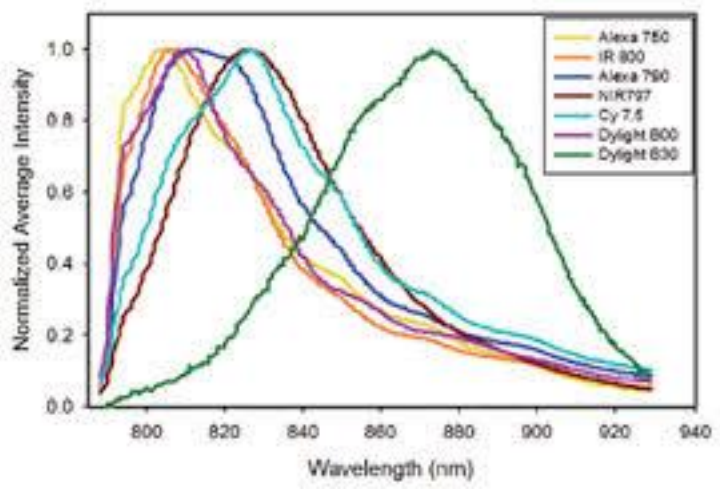

Figure 3: Atomic Force Microscopy slides for looking at build-up and aggregation of IAPP

References/Works Cited

Westermark, P., Andersson, A., & Westermark, G. (2011). Islet Amyloid Polypeptide, Islet Amyloid, and Diabetes Mellitus. *Physiological Reviews*, 795-826. Retrieved October 28, 2015, from http://www.ncbi.nlm.nih.gov/pubmed/21742788

Islet Amyloid in Type 2 Diabetes, and the Toxic Oligomer Hypothesis Leena Haataja, Tatyana Gurlo, Chang J. Huang, and Peter C. Butler (2008) *Endocrine Review*, 29(3): 303–316. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2528855/

Hudson, S., Ecroyd, H., Kee, T., & Carver, J. (2009, August 11). The thioflavin T fluorescence assay for amyloid fibril detection can be biased by the presence of exogenous compounds. Retrieved October 28, 2015, from http://www.laserchemistry.adelaide.edu.au/Kee/Papers_pdf/Thioflavin_T.pdf

Neddenriep, B., Calciano, A., Conti, D., Suave, E., Paterson, M., Bruno, E., Moffet, D.A. (2011). Short Peptides as Inhibitors of Amyloid Aggregation. *The Open Biotechnology Journal*, 39-46.

Baine, M., Georgie, D., Shiferraw, E., Nguyen, T., Nogaj, L., & Moffet, D. (n.d.). Inhibition of Aβ42 aggregation using peptides selected from combinatorial libraries. *Journal of Peptide Science J. Pept. Sci.*, 499-503.

Borman, S. (2010). Attacking Amyloids. *Chemical & Engineering News Chem. Eng. News*, 30-32.

Bruno, E., Pereira, C., Roman, K., Takiguchi, M., Kao, P., Nogaj, L., & Moffet, D. (n.d.). IAPP aggregation and cellular toxicity are inhibited by 1,2,3,4,6-penta- O -galloyl-β- d -glucose. *Amyloid*, 34-38.

Bucciantini, M., Giannoni, E., Chiti, F., Baroni, F., Formigli, L., Zurdo, J., . . . Stefani, M. (2002). Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature,* 507-511.

Chiti, F., & Dobson, C. (n.d.). Protein Misfolding, Functional Amyloid, And Human Disease. *Annual Review of Biochemistry,* 333-366.

Fox, A., Snollaerts, T., Casanova, C., Calciano, A., Nogaj, L., & Moffet, D. (2010). Selection for Nonamyloidogenic Mutants of Islet Amyloid Polypeptide (IAPP) Identifies an Extended Region for Amyloidogenicity. *Biochemistry,* 7783-7789.

Jarvis, L. (2010). The Amyloid Question. *Chemical & Engineering News Chem. Eng. News,* 12-17.

Zelus, C., Fox, A., Calciano, A., Faridian, B., Nogaj, L., & Moffet, D. (2012). Myricetin Inhibits Islet Amyloid Polypeptide (IAPP) Aggregation and Rescues Living Mammalian Cells from IAPP Toxicity. *Open Biochem J The Open Biochemistry Journal,* 66-70. Date Accessed: October 3, 2015.

Cooper, G.J.S., Willis, A., Clark, R.C., Turner, R.B., Sim, K.B., Reid, B.M (1987). Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients (amino acid sequence/calcitonin gene-related peptide/insulin A chain/Alzheimer disease/pancreatic islet). *Proc. National Academy Science USA,* vol. 84.

Haataja, L., Gurlo, T., Huang, C.J., Butler, P.C., (2013). Islet Amyloid in Type 2 Diabetes, and the Toxic Oligomer Hypothesis, *Endocrine Reviews,* v. 29, Issue 3. Date Accessed: September 24, 2015.

Summer Research Budget Outline

Item needing funds	Price ($)
Housing On-Campus	$3000 off-campus residency I will be here May 9-Aug 9, rent @ $1000 a month
IAPP (Human Protein) Samples	$100 each X 20 = $1700
Thioflavin-T dye (Sigma Aldrich) x 2	$49.00
12 x 75mm Small Culture Tubes (American Screening Corp) x 4 @ $34.64	$98.56
Maximum Total Amount Requested	$4,986.12