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Investigating Metal Cations as Potential Inhibitors of IAPP Aggregation: KCl, CaCl2, and CuCl2

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I. Introduction

It is estimated that 25.8 million children and adults in the United States have Diabetes Mellitus, which is roughly 8.3% of the population, and 1.7 new cases are diagnosed each year (Butler, 2003). There are two specific types of Diabetes: Type I and Type II. Type I is genetic, and there is an insulin decrease due to autoantibodies. Type II, which represents 90-95% of the Americans with Diabetes, is adult-onset, also called insulin-resistant or insulin-dependent. As Type II Diabetes progresses, the pancreatic insulin-producing β cells are lost, with up to 45% loss in the most severe cases. There is a theory that the protein Islet Amyloid Polypeptide (IAPP), which is secreted in the pancreas, is involved in the destruction of β cells. It adopts a misfolded structure in a nearly irreversible reaction, aggregates in the pancreas, and becomes toxic, as explained by the toxic oligomer hypothesis (Buccianti, 2002; Haataja et. al, 2009). When IAPP aggregates within the body, it kills β-cells, and the pancreas decreases the release of insulin, causing high, unregulated glucose levels (Neddenriep, 2011). This has a direct correlation to Diabetes and the progression of the disease.

IAPP has become a topic of research in order to combat Type II Diabetes. Recently, zinc chloride in low concentrations was shown to significantly decrease IAPP aggregation by slowing down or even inhibiting fiber formation (Brender et. al, 2010). In this

experiment, three metals (KCl, $CaCl_2$, and $CuCl_2$) were tested with human IAPP to see if they slow down or prevent the aggregation of the protein.

II. Materials and Methods

10mM solutions of the metal cations were prepared in TRIS buffer, and Thioflavin T assays were performed. Thioflavin T (ThT) is a fluorescent dye that binds to aggregated IAPP. 375uL of IAPP was speed vacuumed for 10 minutes to evaporate any liquid from the sample, leaving only the dried amyloid polypeptides (two experimental tubes for the cations, one for IAPP alone). 135uL of TRIS buffer and 15uL of metal cation solution was added to the appropriate experimental tubes, and 150uL of TRIS was added to the IAPP control. They were incubated at 37°C, and at 5 minute intervals, a 17µL aliquot of the sample was added with 663µL of a fluorescent dye, Thioflavin-T, and put into the Fluorescent Spectrophotometer to measure ThT fluorescence.

If the ThT results seemed promising, then Atomic Force Microscopy (AFM) slides were created. AFM uses an ultra-fine needle attached to a cantilever beam, and the needle "feels" the surface of the sample, creating an image of the peaks and valleys. This is useful in visualizing IAPP fibers on a slide, but since it is time consuming, it is only employed when the ThT results showed inhibition of IAPP aggregation.

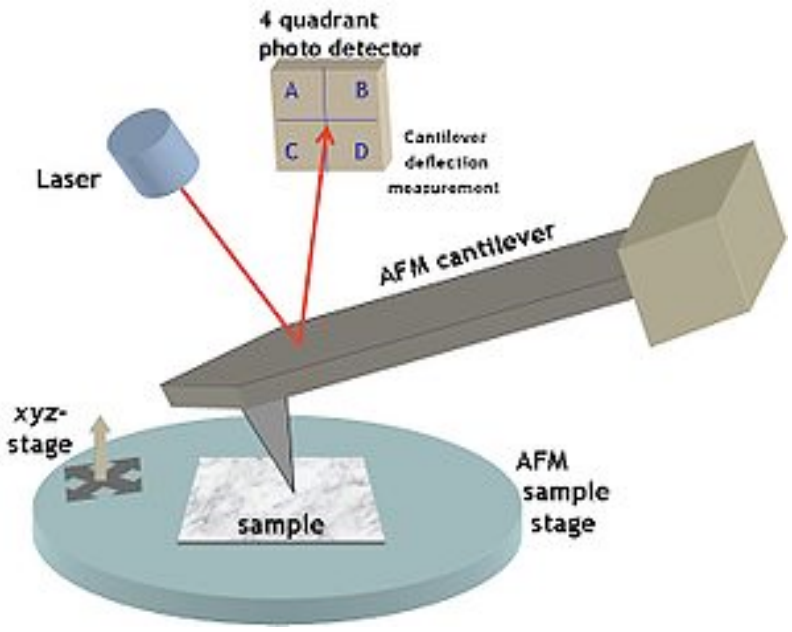

Figure 1: Atomic Force Microscopy (AFM) diagram, including the cantilever, laser, and photo detector.

III. Results

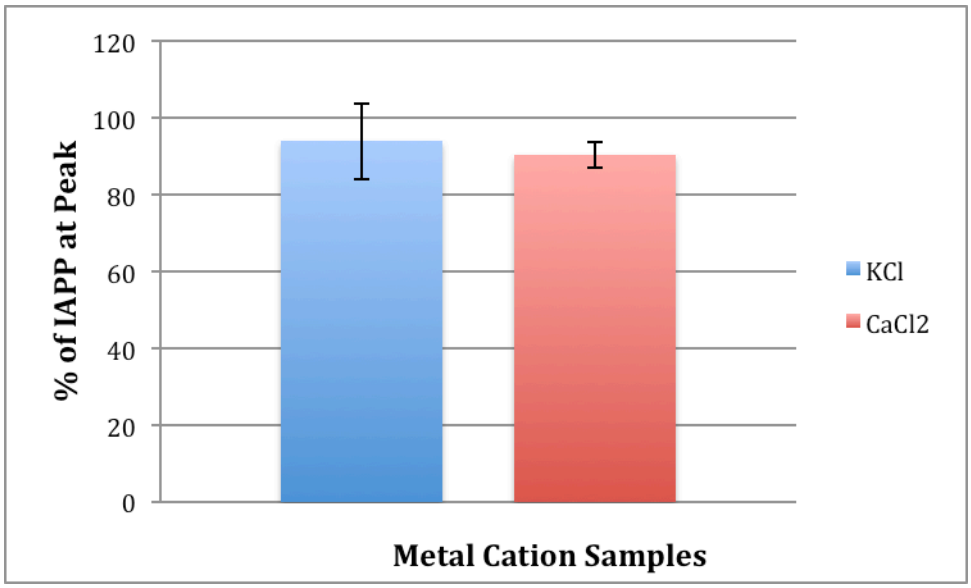

Figure 2: ThT fluorescence of KCl and $CaCl_2$ displayed as percentage of IAPP control at the peak for that trial. Standard deviations are shown as error bars. There was no significant inhibition of IAPP in either KCl or $CaCl_2$.

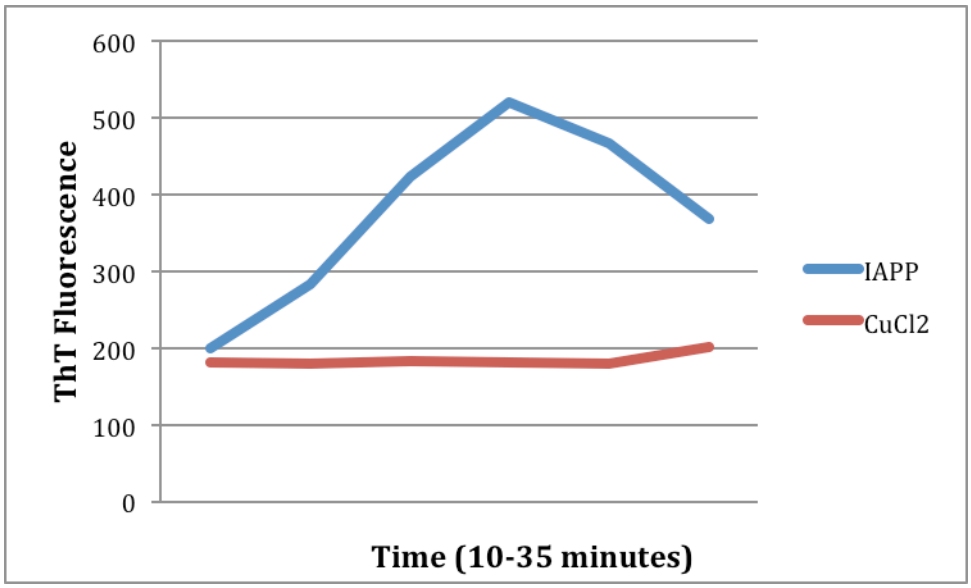

Figure 3: ThT fluorescence of $CuCl_2$ as compared with IAPP control over one trial of 35 minutes. $CuCl_2$ never showed aggregation, and IAPP peaked at 25 minutes.

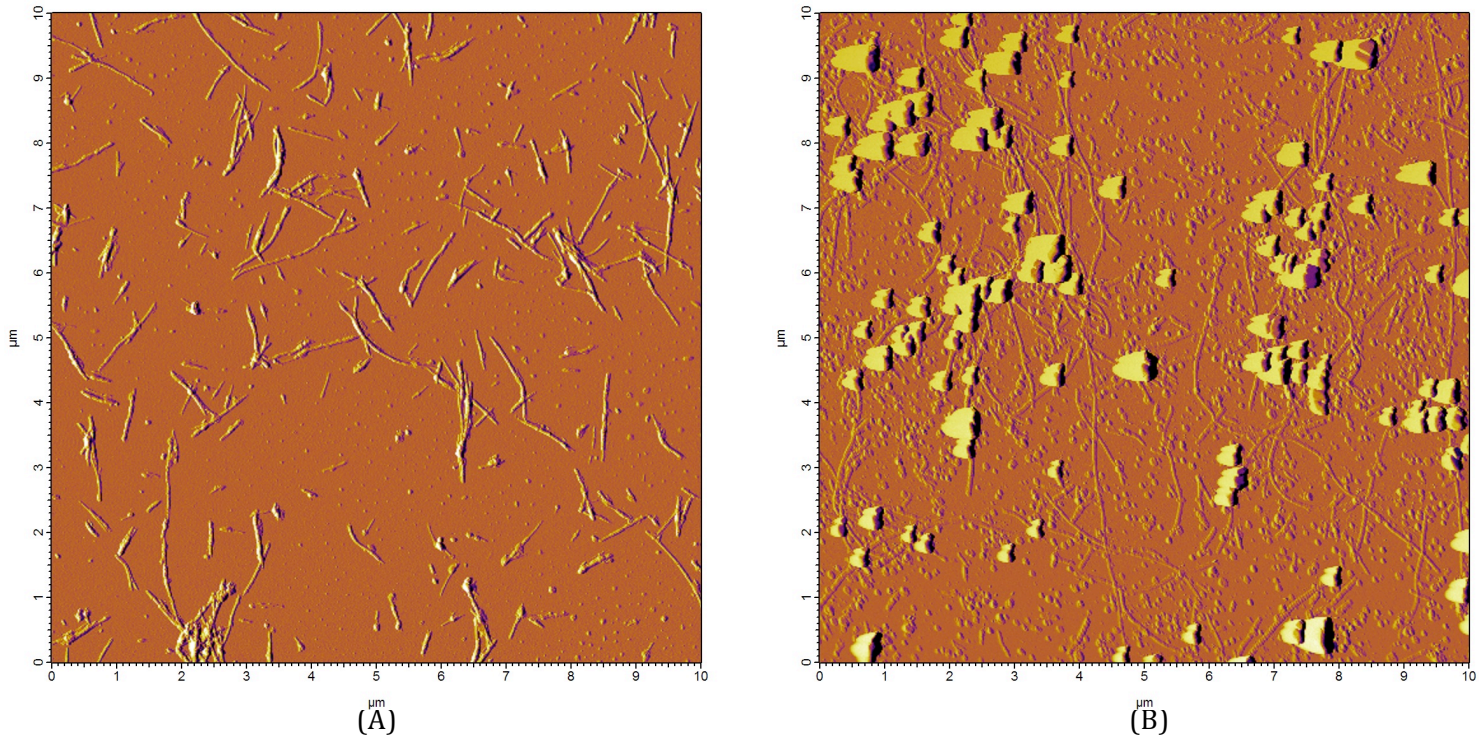

Figure 4: AFM slides of (A) IAPP alone and (B) IAPP with $CuCl_2$. The IAPP alone shows fibers, and the CuCl2 also shows fibers, meaning there was no inhibition of IAPP aggregation, but most likely an interference with ThT fluorescence.

IV. Discussion

KCl (K^+) and $CaCl_2$ (Ca^{2+}) did not show significant inhibition of IAPP aggregation, as seen in Fig. 2. K^+ was 93.86% and Ca^{2+} was 90.22% as compared with the IAPP control, so practically no inhibition occurred. This data was expected, as these metals are not known to inhibit IAPP, but the data was consistent enough to reject these metals as potential therapies for Type II Diabetes.

$CuCl_2$ showed promising results from preliminary ThT fluorescence, as shown in Fig. 3. Over a 35 minute trial, $CuCl_2$ maintained a fluorescence level under 200, and IAPP peaked at 25 minutes at 519.7. Because there was no peak in $CuCl_2$, an AFM slide of the sample was prepared, as shown in Fig. 4. As compared with the IAPP alone, there were still fibers on the $CuCl_2$ slide, so it cannot be concluded that Cu^{2+} inhibited IAPP aggregation and fiber

formation. It has been shown that Thioflavin T can bind to samples instead of IAPP, leading to falsely low fluorescence results and false positives (Hudson *et al*, 2009). This is possibly what occurred with the Cu^{2+}.

For future research, the metal samples could be tested using circular dichromism, which gives real time results as opposed to in intervals, which would prevent the possibility of "missing" the peak in aggregation (McLean & Balasubramaniam, 1992). The samples would also need to be tested on human cell cultures *in vivo* to see if they are plausible for actual treatments of Type II Diabetes Mellitus. Finally, the IAPP sequences between species that get Diabetes and those that do not should be compared, paying particular attention to the SNNFGAILSS region (amino acids 20-29), which causes proteins to fold in manner prone to amyloidogenesis (Westermark *et al*, 1990).

Although this study was not particularly conclusive, it lays the groundwork for future experiments and gives a foundation for further study of metal cations and their interactions with the Type II Diabetes Mellitus protein IAPP.

V. Acknowledgements

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References

Butler, A. E., Janson, J., Bonner-Weir, S., Ritzel, R., Rizza, R. A., & Butler, P. C. (2003). β-cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. *Diabetes*, *52*(1), 102-110.

Brender, J. R., Salamekh, S., & Ramamoorthy, A. (2011). Membrane disruption and early events in the aggregation of the diabetes related peptide IAPP from a molecular perspective. *Accounts of chemical research*, *45*(3), 454-462.

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.

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.

Hudson, S. A., Ecroyd, H., Kee, T. W., & Carver, J. A. (2009). The thioflavin T fluorescence assay for amyloid fibril detection can be biased by the presence of exogenous compounds. *Febs Journal*, *276*(20), 5960-5972.

McLean, L. R., & Balasubramaniam, A. (1992). Promotion of β-structure by interaction of diabetes associated polypeptide (amylin) with phosphatidylcholine. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, *1122*(3), 317-320.

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.

Westermark, P., Andersson, A., & Westermark, G. (2011). Islet Amyloid Polypeptide, Islet Amyloid, and Diabetes Mellitus. *Physiological Reviews,* 795-826.