

# The Effect of a Halogen Ion on Insulin Crystallography

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## Introduction

- ❖ Insulin is composed of two peptide chains, A and B, that are joined together by a disulfide bond.
- ❖ Insulin functions in response to elevated blood glucose levels; it communicates through cell signaling pathways to increase the rate of glucose absorption into muscle and fat cells via Glut4.
- ❖ X-ray crystallography is used to determine the shape of crystals by recording the specific angles and intensities in which the x-rays reflected off the crystal. By analyzing the patterns in the data, scientists can determine the exact structure of the crystal.
- ❖ Crystallization is the technique used to purify solid compounds. This process excludes impurities thus allowing crystals to be filtered from a solution.

## Focusing Question

How does exposure to different halogen ions alter insulin's structure and affect its crystallization?

## Hypothesis

Insulin will maintain the integrity of its structure even when soaked in bromine solutions. When insulin is exposed to these ions, it will change conformation in order to effectively bind to the bromine. This is because proteins have specific conformations in ideal environments, and addition of solutions highlights structural changes. The presence of these halogen ions will thus cause conformational changes in the insulin.

## Discussion

- ❖ As with all proteins, the structure of insulin is crucial to its function in the body. By altering its confirmation, the function of insulin can either be inhibited or enhanced.
- ❖ Studying these changes in insulin's shape can help determine what the ideal form of insulin is in the body. Such studies could potentially increase the efficacy of insulin used in medical treatments.
- ❖ This has a significant impact in the world of medicine; specifically, in regards to diabetes. For those who inject themselves with insulin in order to control their blood sugar levels, a significantly more effective form of insulin would essentially mean an easier way to deal with their illness.

## Materials and Methods

- ❖ 18 mg Insulin
- ❖ 100 uL  $\text{Na}_2\text{HPO}_4$
- ❖ 10 uL Ethylenediamine Tetraacetic Acid
- ❖ 890 uL  $\text{H}_2\text{O}$
- ❖ 400 mM  $\text{Na}_2\text{HPO}_4$  at pH 10.4
- ❖ 10 mM Ethylenediamine Tetraacetic Acid
- ❖ 1:1 Ratio of Protein and Reservoir (2 uL each)
- ❖ 500 mM  $\text{Na}_2\text{HPO}_4$  at pH 10.43
- ❖ Ethylenediamine Tetraacetic Acid Stock, MW = 372.2 mol/g
- ❖ 1 M Sodium Bromide Solution
- ❖ 1 M Sodium Hydroxide Solution
- ❖ 1 M Glacial Acetic Acid
- ❖ 3 M Sodium Chloride (Dissolve 17.53 g NaCl in 100 ml DI-water).
- ❖ 100 mL of 1 M Sodium Citrate (Citric Acid) Buffered to pH 3.5
- ❖ 100 mL of 1 M Sodium Acetate Buffered (with Glacial Acetic Acid) to pH 4.5
- ❖ 100 mL of 1 M Sodium Acetate Buffered (with Glacial Acetic Acid) to pH 5.5
- ❖ 100 mL of 1 M Sodium Phosphate Buffered (with Sodium Hydroxide) to pH 6.5
- ❖ Make 1M solution of NaOH (10mL),  $\text{Na}_2\text{HPO}_4$  buffer at 0.5 mM, pH 10.43 (50mL), and add 40mg mg of NaOH to 10 mL of water
- ❖ Add 40 mL of  $\text{H}_2\text{O}$  to 6.705g of  $\text{Na}_2\text{HPO}_4$
- ❖ Titrate the solution with 1 N  $\text{NaOH}_2$  to a pH of 10.43 and dilute with water until final solution is 50 mL
- ❖ From the insulin stock solution, prepare the 24 reservoir solutions for the crystallization experiments and pipette 0.5 ml of the corresponding reservoir solution into each of the 24 reservoir wells of a Cryschem™ plate
- ❖ Pipette 1  $\mu\text{l}$  of the resulting reservoir solution into the crystallization cup on the sitting drop post in ekkach well
- ❖ Store the plate at 20 °C. The crystals will start to grow immediately in some wells, and growth can be clearly observed after a 1 week growth period.
- ❖ Let crystals grow for 1 week and then soak in NaBr2 solution for 5 minutes

## Conclusion

The results that were collected at Argonne National Laboratories, show that bromine is capable of binding to insulin and altering insulin's structure.

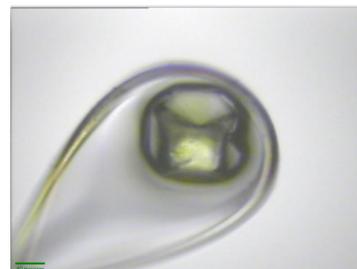
## Further Studies

The structural study of insulin can be furthered by introducing more environmental variables, such as temperature to discover the optimal conditions for the efficiency of insulin.

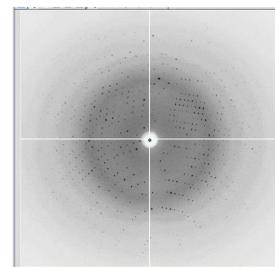
## Applications

- ❖ Examining the bonding habits of insulin gives a greater understanding of insulin and its ability to function in different environments.
- ❖ Uncovering the depth of insulin's structure can allow scientists to target and discover more effective measures of treatment for diabetes.

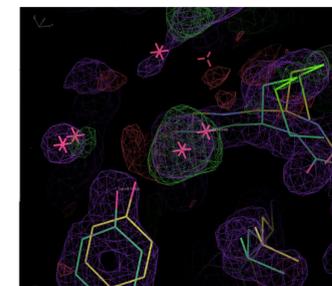
## Results



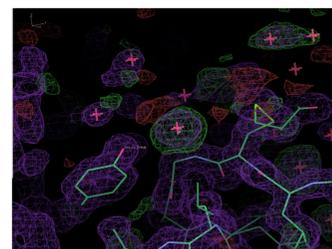
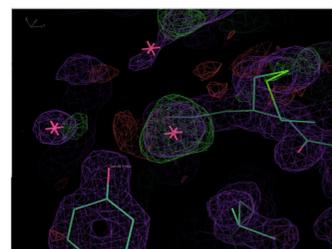
Visualization of Crystal



Insulin X Ray Crystallography



Comparison of the Structures of Insulin and Insulin Soaked in Bromine



Examples of the Structure of Insulin Soaked in Bromine

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