**The Effects of Varying Relative Humidity on the Crystal Structure of Lysozyme**

Jacqueline Chen and Reed Smith  
Florida State University Young Scholars Program  
Sponsored by: Dr. Thayumanasamy Somasundaram  
Institute of Molecular Biophysics

---

**Introduction**

In order to study the structures of proteins, the molecules themselves must first become organized in such a way that they can be measured. The simplest way to produce organized, pure, protein molecules is through the synthesis of a crystal. In this process of protein crystallization, the smallest structural unit of a crystal is known as the unit cell. The arrangement of these unit cells within the crystal is known as the crystal’s space group. As visible light has a wavelength in the hundreds of nanometers, it cannot be used to measure proteins, which generally are found in units of angstroms, or 0.1 nanometers. Meanwhile, X-rays have comparable wavelengths to that of proteins and therefore are an effective means of analysis. X-ray diffraction involves firing a concentrated beam of X-rays at a sample protein crystal and recording the resulting scatter on an image plate. The structure of the protein crystal can be determined by calculating the intensities of this scattering and at various angles.

Within each structural unit of the crystal, the protein molecules are bonded together in a certain configuration. One of the common ways for this bond to be achieved is through indirect hydrogen bonds with one or more molecules of water and the other way is via crystal contacts. Water (bulk) is also present in solution in the space between protein molecules. To determine whether varying relative humidity will correlate to a change in the cell dimensions of the crystal structure of hen egg white lysozyme using X-ray diffraction, the researchers decided to conduct experiments to find out if such a correlation exists.

**Research Goals**

- Determine whether varying relative humidity will correlate to a change in the cell dimensions of the crystal structure of hen egg white lysozyme using X-ray diffraction.
- Gain experience in the field of X-ray crystallography.
- Learn the techniques used to analyze protein structure and composition.
- It is predicted that a positive correlation will be found between relative humidity and cell dimension.

**Methods**

**Crystal Mounting**

- High quality single crystals were selected from a cover slips on the VDX plate. The slip was carefully removed without disturbing the drops and the crystals were transferred to individual drops of the well solution using a nylon loop.
- The crystals were then mounted in 1.0 mm diameter glass capillaries and all excess solution was removed using a filter paper wick.
- In the other end, a small amount of one of the six saturated solutions was injected. This process was repeated for the other five solutions and once for the well solution.
- In each capillary, the crystal and the solution were kept separate.
- After allowing the closed system to equilibrate over several days, this process will create several different relative humidities inside each capillary.

**Solution Preparation**

- A sodium acetate buffer was prepared at pH 4.8. This buffer maintains a constant pH environment to protect the lysozyme.
- A 10% by v/v sodium chloride precipitant solution was prepared. This addition of sodium chloride causes lysozyme to precipitate, forming crystals.
- To maximize the quantity of unstable single crystals, four lysozyme solutions of 40, 30, 20 and 10 mg/mL, concentrations were prepared in the sodium acetate buffer. Each was filtered using an Acrodisc syringe filter to ensure the sterility of the lysozyme solution.
- Six saturated solutions of the following salts were prepared: lithium chloride, magnesium chloride, sodium chloride, potassium chloride, sodium bromide, and disodium phosphate.

**Crystallization through Hanging Drop Method**

- Each well of a 4 x 6 pre-greased VDX plate was filled with 0.6 mL of the 10% sodium acetate precipitant solution.
- On a siliconized glass cover slip, two 3 µL drops of lysozyme solution were placed. Each drop was mixed with another 3 µL of sodium chloride precipitant solution.
- A similar slip was placed drop side down over each well in the plate with each row of the plate corresponding to a different concentration of lysozyme solution.
- Over a period of approximately four days, the protein solution becomes super saturated, allowing for crystal growth.

**Examination of Crystal Growth**

- The VDX plate was examined under a light microscope for crystal growth.
- A slip was placed drop side down over each well in the plate corresponding to a different concentration of lysozyme solution.
- The crystals were then mounted in 1.0 mm diameter glass capillaries and then integrated with the remaining 39 frames. From this process, cell dimension values were obtained.

**Results**

<table>
<thead>
<tr>
<th>Salt Solution</th>
<th>Magnesium Chloride</th>
<th>Sodium Chloride</th>
<th>Disodium Phosphate</th>
<th>Standard Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Humidity (% at 25°C)</td>
<td>33</td>
<td>75</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>a = 78.488</td>
<td>a = 78.638</td>
<td>a = 79.944</td>
<td>a = 79.540</td>
<td></td>
</tr>
<tr>
<td>b = 78.488</td>
<td>b = 78.944</td>
<td>b = 78.540</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c = 38.383</td>
<td>c = 38.425</td>
<td>c = 38.268</td>
<td>c = 37.770</td>
<td></td>
</tr>
<tr>
<td>Cell Dimensions (Å)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α = 90</td>
<td>β = 90</td>
<td>γ = 90</td>
<td>α = 90</td>
<td></td>
</tr>
<tr>
<td>Cell Angles (°)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α = 90</td>
<td>β = 90</td>
<td>γ = 90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The crystals of all three solutions were indexed for a tetragonal space group.

Matthew’s Coefficient Constant: 2.04 Å³ / Da  
Water Content: 39.6%

**Conclusion**

A trend was observed among the three crystals that were diffracted successfully. Crystals in capillaries with lower relative humidity tended to have smaller cell dimensions than crystals in capillaries with higher relative humidity. The difference, however small, may have been a result of water molecules being removed from the crystal structure as the system attempts to go to equilibrium. This change could also simply be the result of experimental error.

Future experimentation would involve testing a larger sample size, a wider range of relative humidity, and a variety of protein samples in order to determine if bulk loss is the cause of the difference in cell dimensions.

**Acknowledgments**

The researchers would like to thank the Young Scholars Program for providing the opportunity to conduct independent research, Dr. Claudius Mwundeni, Dr. Joan Hare, and especially Dr. Thayumanasamy “Soma” Sundaram for the care and special attention he dedicated to this research.

---

**Lysozyme Circular Dichroism**

**X-Ray Diffraction**

The capillary was centered in the X-Ray beam of the R-Axis IV++ image plate detector (Rigaku) and a copper rotating anode source. Diffraction data were collected at a wavelength of 1.541 Å with 40 frames of 1° oscillation and 6 minutes of exposure per frame. A frame was chosen at random and using the HKL Suite was indexed and then integrated with the remaining 39 frames. From this process, cell dimension values were obtained.