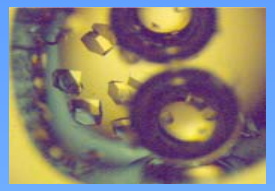
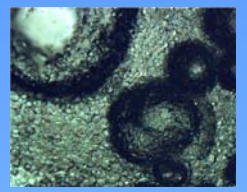


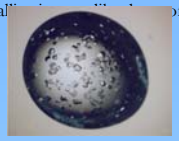
Crystallization, X-Ray Diffraction and Circular Dichroism of Chicken Egg-White Lysozyme

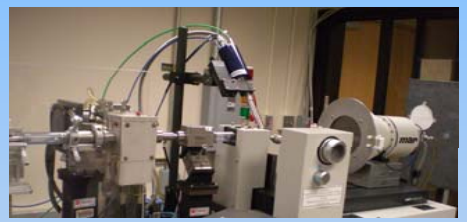
A. Kirsch and L. Chung
Young Scholars Program 2009
Sponsored by Dr. Thayumanasamy Somasundaram



Basics and Structure

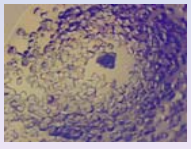
Lysozyme are a family of enzymes that are found in human mucus, saliva and in chicken egg-white. Lysozyme was discovered by Alexander Flemming in 1922. in 1965, David Chilton Phillips determined the three –dimensional structure of Chicken egg-white Lysozyme using x-ray diffraction, making it the first enzyme to have its structure determined by x-ray diffraction. Lysozyme is now commonly used for X-Ray analysis because it is easy to purify from egg-whites. Its properties also allow for efficient crystal[illegible]of other proteins.

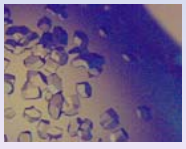




Preparation and Crystallization

In order for the lysozyme enzyme to undergo x-ray diffraction, the appropriate crystals must be obtained utilizing the hanging drop method. First, a 53.6 mg/ml sample of lysozyme in 0.1 M of NaAc, with a pH of 4.8 was prepared. Next, a precipitate buffer consisting of 9.75g NaCl and 150 mL of 0.1 M NaAc with pH 4.8 was made. Using a VDX tray, 600 mL of the precipitant buffer was placed into each well. Then, cover slips with a mixture of 5 microliters of the precipitant buffer and 5 microliters of the lysozyme sample were situated over the wells. Each of the four trays hold different concentrations of lysozyme: S1 (53.5 mg/mL), S2 (26.8 mg/mL), S3 (13.4 mg/mL), S4 (6.7 mg/mL).

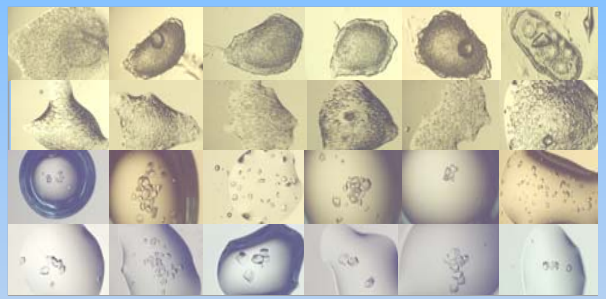




Overview

To appreciate the function of a protein and how it performs this specific function, scientists need to understand its structure. In order to formulate the structure of the chicken egg-white lysozyme protein, the enzyme was crystallized then underwent x-ray diffraction. In addition, the lysozyme samples were analyzed using a circular dichroism spectrometer to identify their composition. Analyzing the crystals under various temperatures in hopes of better comprehending the properties of the protein with respect to the temperature, it is hypothesized that as the heat of the enzyme increases, the internal structure of the enzyme will also increase in size .

Data

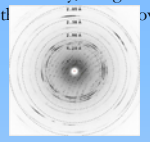


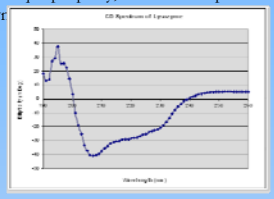
The first row contains crystals made from the S1 concentration. The second row contains S2 crystals, the third row contains S3 crystals, and the fourth row contains S4 crystals.

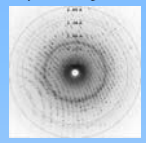
Statistical Table	
Data Collection Range (Å):	60 Å –1.9 Å
Collection (degree):	180° (120 x 1.5 °) 90 ° (60 x 1.5 °) 90 ° (45 x 2 °)
Redundancy:	5-8 times
Completion:	94-97%
R-factor:	6.5-8.8%
I/ σ :	23

X-Ray Diffraction and Spectroscopy

To determine the internal structure of the crystals, x-ray diffraction was used, in which beams of x-rays are shot through a crystal. When x-ray beams strike the crystal, light scatters, causing a diffraction pattern to appear. The crystal is placed in a tiny loop and flash cooled in liquid nitrogen to reduce the damage of radiation on the crystal , thus allowing the crystal to be observed at various temperatures. Circular dichroism Spectroscopy was also used to determine the internal structure of lysozyme. Circular Dichroism is observed when optically active matter, such as, the lysozyme solution, absorbs circular polarized light. All matter absorbs the light differently; using this unique property, one can compare the lysozyme samples to th[illegible]owr







Results and Discussion

	Crystal 1	Crystal 2	Crystal 3	Average Size for Each Temperature
90°	78.72	78.38	77.23	78.11
130°	78.80	78.42	77.33	78.25
170°	78.92	78.52	77.42	78.29

It was observed that as the temperature increased, the average size of the crystal for each temperature increased as well. The pattern was also observed in crystals 1, 2 and 3. Each of the three crystals have slightly different dimensions for a given temperature. This is to be expected, as the internal structure of every crystal varies.

Conclusion

Based on the experimentation and results, it is determined that the internal structure of lysozyme does expand as the temperature increases. While the results showed evidence of the correlation, more data is needed to prove this connection. In future studies, this phenomenon can be analyzed using other proteins and enzymes with varying internal structure shapes and sizes.

We would like to thank the FSU Young Scholars Program for providing us with the opportunity to conduct this research. We are grateful for the help of Dr. Claude Mundoma for allowing us to use the Physical Biochemistry Facility and for his time. We would especially like to thank our sponsor, Dr. Thayumanasamy Somasundaram, for allowing us the use the X-Ray Crystallography Facility, guiding us through our research, and teaching us about the world of scientific study.