Crystallization and x-ray diffraction of chicken egg-white lysozyme

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Basics and Structure

The enzyme lysozyme, named *lyso* for its ability to lyse bacteria, was discovered by Alexander Fleming in 1922 during his search for an antibiotic. Originally discovered in nasal mucus, other bodily secretions and egg whites have since been found to be a rich source of lysozyme as well. Lysozyme functions as a mild antibiotic by breaking down bacterial cell walls. In eggs, it ensures the development of chicks through the protection of proteins and fats necessary for their nourishment.

The three-dimensional structure of lysozyme was first determined in 1965 by David Phillips and his colleagues. Chicken egg-white lysozyme, a fairly small and compact enzyme, is a single polypeptide chain of 129 residues. It is highly stable and is cross-linked by four disulfide bridges. Lysozyme occupies an ellipsoidal shape with dimensions of roughly 45 x 30 x 30 Å and possesses a complex folding structure. It consists of alpha (α) helixes and contains a β-sheet structure in various areas along the polypeptide chain.

Preparation and Crystallization

Lyophilized chicken egg white lysozyme was crystallized using the common hanging drop method, where drops of protein stock and mixed with drops of buffer solution on a cover slip. The cover slip was then flipped so that the drop was suspended above a well of the same buffer solution, with a ring of vacuum grease sealing off the drop from the outside. Because the concentration of buffer was lower in the hanging drop than in the well, more water molecules moved from the drop to the reservoir than in the other direction in order for the system to reach equilibrium. As water molecules left, the dissolved lysozyme slowly began to crystallize.

Batch crystallizations were performed in 24 well VDX plates with pre-applied sealant at room temperature using a buffer solution consisting of 6.5% (w/v) NaCl in 0.1 M NaAc pH 4.8. NaCl was added because water molecules are more attracted to NaCl than to lysozyme, forcing lysozyme molecules to bind with each other rather than to water molecules.

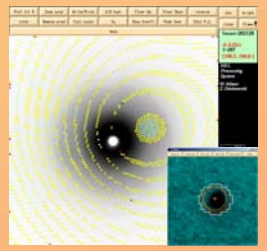
Left: Lysozyme crystal mounted in a rayon loop
Right: Mounted crystal under the CryoStream

Overview

In this study, chicken egg-white lysozyme was crystallized using a buffer solution of sodium chloride in a sodium acetate solution. Using the widely known hanging drop method, lysozyme crystals were grown and later prepped for study by flash-cooling with liquid nitrogen. Using the method of x-ray diffraction data was collected to study the variation in the cell dimensions of different crystal sizes.

It was originally hypothesized that larger crystals would have larger cell dimensions while smaller crystals would have smaller cell dimensions based on the idea that large objects freeze slower than small objects. It was believed that the differences in freezing time would have a effect on the cell dimensions of the crystals. However, after running three trials on large crystals and another three trials on small crystals, it was found that the freezing rate of the crystals, big or small, was uniform and therefore did not affect the cell dimensions. Thus, the hypothesis was proved to be incorrect.

Top: R-Axis IIc, an image plate detector
Bottom: Analysis of x-ray diffraction

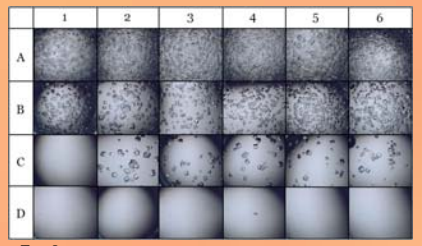


Data

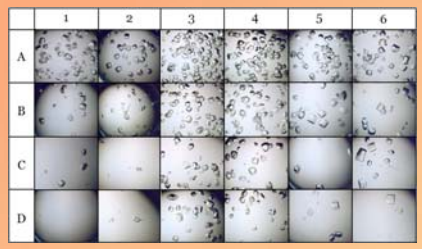
	1	2	3	4	5	6
Drop Size:	6 µl	6 µl	6 µl	6 µl	3 µl	3 µl
A	75 mg/mL	75 mg/mL	75 mg/mL	75 mg/mL	75 mg/mL	75 mg/mL
B	37.5 mg/mL	37.5 mg/mL	37.5 mg/mL	37.5 mg/mL	37.5 mg/mL	37.5 mg/m
C	18.75 mg/mL	18.75 mg/mL	18.75 mg/mL	18.75 mg/mL	18.75 mg/mL	18.75 mg/mL
D	9.375 mg/mL	9.375 mg/mL	9.375 mg/mL	9.375 mg/mL	9.375 mg/mL	9.375 mg/mL

	1	2	3	4	5	6
Drop Size:	12 µl 6 µl stock protein; 6 µl NaAc	12 µl 6 µl stock protein; 6 µl NaAc	12 µl 9 µl stock protein; 3 µl NaAc	12 µl 9 µl stock protein; 3 µl NaAc	12 µl 7.2 µl stock protein; 4.8 µl NaAc	12 µl 7.2 µl stock protein; 4.8 µl NaAc
A	37.5 mg/mL	37.5 mg/mL	37.5 mg/mL	37.5 mg/mL	37.5 mg/mL	37.5 mg/mL
B	25 mg/mL	25 mg/mL	25 mg/mL	25 mg/mL	25 mg/mL	25 mg/mL
C	18.75 mg/mL	18.75 mg/mL	18.75 mg/mL	18.75 mg/mL	18.75 mg/mL	18.75 mg/mL
D	15 mg/mL	15 mg/mL	15 mg/mL	15 mg/mL	15 mg/mL	15 mg/mL

Bottom: View of crystals in the crystal trays



Tray 2



Tray 3

X-ray Diffraction and Cryocrystallization

In order to probe an object as small as a protein molecule, one requires a probe capable of distinguishing atoms. Thus the x-ray, with its 10 to 0.1 nanometer wavelength, is the optimal candidate for protein structure investigations. When an x-ray beam hits a protein crystal, the electrons in the lattice scatter the x-rays into a distinct pattern. However, one scattering pattern is not enough—the crystal must be rotated slowly, with scatterings collected for a 180° range. These 2D scattering images are then translated into a single 3D electron density map using the mathematical method known as Fourier Transforms. However, creating an electron density map is beyond the scope of this project.

Unfortunately, the incident x-rays contain so much energy that they begin to heat up the sample, degrading the protein. Thus, freezing the crystal is necessary in order to preserve the structure of the crystal. However, conventional freezing freezes the crystal too slowly and unevenly, oftentimes dropping or destroying the fragile crystal from the micro loop in the process. Because of this the use of liquid nitrogen has become more common within the past decade. This "flash cooling" can be done by simply dunking the entire micro loop with the crystal into a bowl of liquid nitrogen, but this requires extensive transfer procedures, to ensure that the crystal does not melt by the time it is mounted on the machine. A simpler method is to simply freeze the crystal when it is already mounted on the x-ray diffraction machine using a device called the Oxford CryoStream. For the entire duration of the diffraction collection process—which takes hours to complete—the crystal is kept at 100 K by this device in the loop suspended in the machine's stream of freezing gaseous nitrogen.

Results and Discussion

The ratio between precipitant and protein was altered as well, with more lysozyme added in columns 3, 4, 5, and 6 of Trays 3 and 4, with columns 1 and 2 acting as the control with their equal portions of precipitant and protein. No discernible differences were noticed between the crystal sizes grown in the respective columns.

After growth of the crystals, three small and three large were selected for x-ray diffraction. The data processed by the diffraction software found the cell dimensions to be as follows for the six crystal trials:

Date	Size	A	B	C
7/10/08 – 7/11/08	Small	79.251 Å	79.251 Å	36.972 Å
7/11/08 – 7/12/08	Small	78.062 Å	78.062 Å	37.056 Å
7/16/08 - 7/17/08	Small	78.557 Å	78.557 Å	36.999 Å
	Avg:	78.623 Å	78.623Å	37.009Å

Date	Size	A	B	C
7/1/08 – 7/2/08	Large	79.686 Å	79.686 Å	36.950 Å
7/8/08 – 7/9/08	Large	77.823 Å	77.823 Å	37.086 Å
7/9/08 – 7/10/08	Large	78.611 Å	78.611 Å	36.898 Å
	Avg::	78.707 Å	78.707 Å	36.978 Å

Conclusion

While one might assume that larger lysozyme crystals would cool at a slower rate than smaller ones, thus disrupting the cell dimensions, evidence shows that unit cell dimensions are not significantly affected by the different crystal sizes.

However, for a more secure claim, more diffraction trials are necessary.

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