The Crystallization of Hen Egg White Lysozyme

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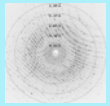
Background Information

Lysozyme is found in human tears and mucus and is also in hen egg whites. It protects against bacterial infection by breaking down the bacterial cell walls. In eggs it protects proteins and fats, necessary nourishment for the chick. Lysozyme used in experiments is from hen egg whites because it appears in such large concentrations and is easy to harvest. This protein would have great potential as an antibiotic if not for its large size.

A common method for the preparation of lysozyme crystals is the hanging drop technique. In this procedure the wells of a microplate are filled with a mother liquor (prepared from buffer and precipitant). A drop made up of lysozyme and the well solution is suspended from a siliconized cover slip over the well, which is then sealed shut with a layer of grease. The difference between the well solution concentrations in the drop and in the well creates a transfer of water from drop to well and vice versa until equilibrium is reached. After a few days crystals will form on the cover slip.



Left: Lysozyme crystal mounted in a rayon loop

Right: Diffraction pattern for a lysozyme crystal.

X-Ray Diffraction

An X-Ray is a type of electromagnetic radiation. In crystal diffraction a beam of X-Rays are aimed at a crystal using three planes of adjustment. The beam is then shot as the crystal slowly rotates. The diffracted X-Rays are absorbed and the pattern of diffraction is printed out onto a computer screen. From this diffraction pattern an electron density diagram can be created, and from this the structure of a macromolecule such as lysozyme can be determined. The problem with this technique is that the X-Rays are so penetrating that they actually destroy the crystal over time. This occurs very quickly when using a small crystal. The speedy degradation of a small crystal is why crystallographers try to create a single large crystal.

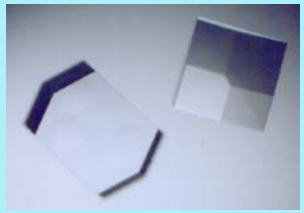
In the past crystals were painstakingly mounted in a capillary and then the X-Ray was shot through the capillary at room temperature. The capillary technique of crystal mounting introduces new mediums for the X-Ray to travel through, therefore distorting the diffraction pattern. To avoid this, rayon loops are used to mount crystals. The well solution and polyethylene glycol (a solution with a function similar to antifreeze) are mixed and the crystal is caught in the loop. This loop is mounted on the X-Ray machine. Crystals are better observed while frozen, so as to minimize radiation damage. To avoid the formation of ice, which would distort the diffraction pattern, the crystal is flash cooled with gaseous nitrogen.

Preparation of Solutions

A 0.1 M sodium acetate buffer with pH 4.6 was prepared by dissolving 5.4432g of sodium acetate in 400ml of distilled water. The pH was adjusted using aliquots of concentrated hydrochloric acid. A 10% weight per volume sodium chloride precipitant was prepared by adding 5g of sodium chloride to 50ml of the buffer. The pH was adjusted to 4.2 using hydrochloric acid and sodium hydroxide.

The initial stock protein (used in tray one and two) was prepared by mixing 100mg of lysozyme in 2ml of distilled water. This protein solution was then vortexed to stir the solution and filtered to remove all impurities. The stock protein was serially diluted to 17.5mg/ml, 5mg/ml, and 1.5mg/ml and these dilutions were stored in three microfuge tubes.

For the stock protein used in trays three through six, 80mg/2ml and 120mg/2ml were used in addition to the initial 100mg/2ml stock protein.



Description of Project

Crystallization is used to obtain the structure and therefore function of a macromolecule. This technique is most often used with proteins. In the course of this project hen egg white lysozyme was crystallized with the hanging drop method and the crystals were then analyzed to determine the optimal conditions for crystal growth. Many trials were conducted with varied conditions to achieve this purpose. Some crystals were then exposed to a high-intensity X-Ray to obtain the diffraction pattern. If it were not for time constraints, the electron density diagram and the structure of the crystals could then have been determined.

Conclusion

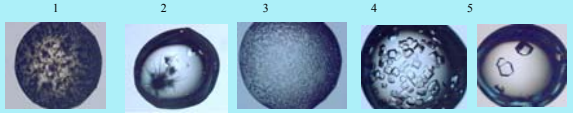
The optimal temperature for the formation of hen egg white lysozyme crystals was found to be room temperature (approximately 22.5 degrees Celsius). The best sodium chloride precipitant to sodium acetate buffer ratio was found to be 0.65ml to 0.35ml. A drop size of 10µl of 50mg/ml lysozyme and 10µl of well solution contributed to optimal crystal growth.

The optimal crystal size for X-Ray diffraction was found to be a large crystal because it can withstand the high energy X-Ray radiation for a much longer time than a smaller crystal.

Importance of Research

The natural functions of lysozyme have created an interest in its scientific potential. The functions of an enzyme are directly related to its structure; to obtain a better understanding of lysozyme, its structure must be determined through procedures such as ours. This experiment has laid the groundwork for determining the structure of this type of lysozyme (Mucopeptide N-acetylmuramdylhydrolase). The development of better crystals using the determined optimal conditions allows for a more accurate diffraction pattern and structural map, as the optimized crystals will not degrade as quickly during X-Ray diffraction.

Data



Pictures 1 and 2: Needle clusters inhibit the growth of crystals.

Picture 3: Hundreds of miniscule crystals form at 4 degrees Celsius.

Picture 4: Many small crystals form, preventing the growth of a few large crystals.

Picture 5: A few large crystals, the desired result.

Tray 1 & 2

Row A: Stock protein 50mg/ml
Row B: Stock protein 17.5mg/ml
Row C: Stock protein 5mg/ml
Row D: Stock protein 1.5mg/ml
Columns 1 & 4: 1ml precipitant well solution
Columns 2 & 5: 0.65ml precipitant and 0.35ml buffer well solution
Columns 3 & 6: 0.5ml precipitant and 0.5ml buffer well solution
Drop size a constant 10µl lysozyme and 10µl well solution. At room temperature.

Tray One

	1	2	3	4	5	6
A	Needle clusters, lots of crystals	2 crystals	----------	Needle clusters, lots of crystals	1 crystal	----------
B	1 tiny crystal, lots of needle clusters	----------	----------	1 tiny crystal, lots of needle clusters	----------	----------
C	----------	----------	----------	----------	----------	----------
D	----------	----------	----------	----------	----------	----------

Tray Two

	1	2	3	4	5	6
A	1 needle cluster, medium amount of crystals	1 crystal	----------	Medium amount of crystals	4 crystals	----------
B	Several needle clusters	----------	----------	Lots of needles	----------	----------
C	----------	----------	----------	Some crystals and needle clusters	----------	----------
D	----------	----------	----------	----------	----------	----------

Tray 3 & 4

Row A: Drop size 10µl protein, 10µl well solution
Row B: Drop size 7.5µl protein, 10µl well solution
Row C: Drop size 5µl protein, 10µl well solution
Row D: Drop size 2.5µl protein, 10µl well solution
Columns 1 & 4: 60mg/ml stock protein
Columns 2 & 5: 50mg/ml stock protein
Columns 3 & 6: 40mg/ml stock protein
Well solutions consist of 0.65ml precipitant and 0.35ml buffer. At room temperature.

Tray Three

	1	2	3	4	5	6
A	Some needle clusters, 29 crystals	6 large crystals	14 medium crystals	17 large crystals and 1 small	9 large crystals	17 medium crystals
B	30 medium crystals	5 medium crystals	9 medium crystals	45 medium crystals	6 medium crystals	15 medium crystals
C	54 medium crystals	1 medium crystal	5 medium crystals	30 medium crystals	3 medium crystals	2 medium crystals
D	34 small crystals	----------	----------	5 small crystals, 1clump	----------	----------

Tray Four

	1	2	3	4	5	6
A	30+ small crystals	5 large crystals	13 crystals of varied size	40+ small, clumping crystals	1 small, 2 large crystals	11 medium and small crystals
B	20+ small, some clumping crystals	13 medium-large and small crystals	20+ total. Lots of small, clumping on the side, some medium crystals	30+ small and medium crystals	2 large crystals	1 large and 1 medium crystal
C	20+ small-medium crystals	1 small and 2 medium crystals	1 small crystal	30+ small crystals, 3 needle clusters	1 large, 1 medium, and 1 small crystal	6 medium crystals
D	35+ tiny crystals	----------	35+ tiny crystals	----------	----------	----------

Tray 5 & 6

Row A: 10µl protein solution, 10µl buffer
Row B: 5µl protein solution, 10µl buffer
Columns 1 & 4: 60mg/ml stock protein
Columns 2 & 5: 50mg/ml stock protein
Columns 3 & 6: 40mg/ml stock protein
Well solutions consist of 0.65ml precipitant and 0.35ml buffer.

Tray 5 is at 16.4 degrees Celsius.
Tray 6 is at 4 degrees Celsius.

Tray Five

	1	2	3	4	5	6
A	Lots of little crystals	15 crystals total. Some medium and small crystals	20+ small crystals, more spread out	A great deal of small crystals (more than A1)	20 medium crystals	30 crystals total. Medium crystals mixed with small
B	A lot of small crystals (especially on the edge)	8 crystals total. Most are medium, a few are small	15 medium crystals	A lot of small crystals	Large needle cluster, 5 small crystals	20 small crystals

Tray Six

	1	2	3	4	5	6
A	Hundreds of very tiny crystals	Hundreds of very tiny crystals (a little less than A1, more spaced)	Hundreds of very tiny crystals (a little less than A2, more spaced)	Hundreds of very tiny crystals	Hundreds of very tiny crystals (a little less than A4, more spaced)	Hundreds of very tiny crystals (a little less than A5, more spaced)
B	Hundreds of very tiny crystals	Hundreds of very tiny crystals (a little less than B1, more spaced)	Hundreds of very tiny crystals (a little less than B2, more spaced)	Hundreds of very tiny crystals	Hundreds of very tiny crystals (a little less than B4, more spaced)	Hundreds of very tiny crystals (a little less than B5, more spaced)

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