Name: Date:

HANDS-ON LAB

Exploring Protein Crystallization

BACKGROUND

Most genes code for proteins. Proteins fold into specific three-dimensional (3D) structures that are held together by interactions between amino acids in the protein. If the structure changes, the protein will not function properly. Knowing the 3D structure of a protein provides some insight into how the protein works.

One technique to determine protein structure is called x-ray crystallography. First, protein crystals are formed from a solution. The crystals are produced when the molecules solidify in regular, repeating geometric patterns, like snowflakes and diamonds, for example. The protein crystals are then exposed to x-rays, which change direction and properties when they hit the crystal. Researchers use this information to make 3D computer models of the protein.

To make protein crystals in the lab, a saturated protein solution is prepared in a buffer. Water moves out of the solution, which encourages the formation of crystals. In the "hanging drop" method of crystallization, a solution called a precipitant is added to the saturated protein solution. A drop of the protein/precipitant solution is placed in a sealed chamber over a reservoir of precipitant in solution. Over time (days to several weeks) crystals develop in the drop. In this activity, you will attempt to form crystals from the protein lysozyme, an enzyme that is abundant in human tears. You will add salt as the precipitant and determine the salt concentration that is needed to form large, highly-ordered protein crystals.

MATERIALS

- balance
- beaker, 100 mL (5)
- crystallization buffer, 25 mL
- graduated cylinder, 10 mL (4)
- grease
- labeling tape
- lysozyme, 0.2 g
- markers
- micropipette, 1–5 μl
- micropipette tips
- pipette, 1 mL
- round cover slip (4)
- sodium chloride (NaCl)
- 24-well plate

PROCEDURE

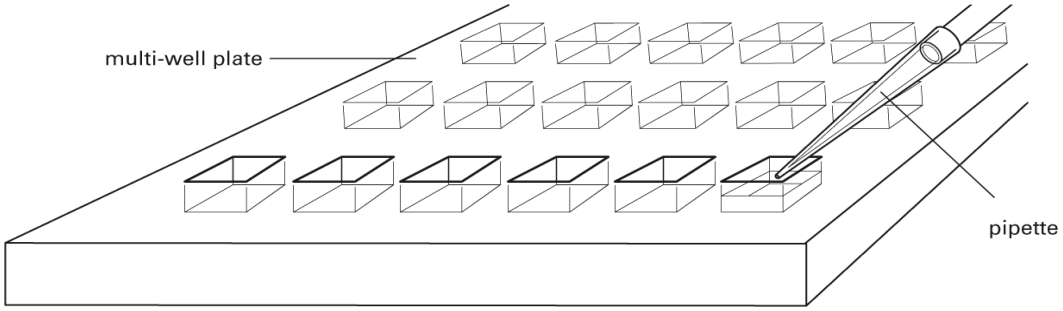
1. Measure 5 mL crystallization buffer and put it into a small beaker.
2. Weigh out 0.2 g lysozyme.
3. Add the lysozyme to crystallization buffer. Leave it alone for 5 to 10 minutes to let the lysozyme dissolve in the buffer. Very gently tap the beaker if the lysozyme is not dissolving.
4. Use tape and a marker to label the remaining Beakers 1–4, and then add 5 mL crystallization buffer to each of these beakers.
5. Determine how much NaCl you need to add to the crystallization buffer in the beakers and record this in Data Table 1. Beaker 1 should contain a solution with 2% NaCl (weight per volume), Beaker 2 should contain a solution with 3% NaCl, Beaker 3 should contain a solution with 5% NaCl, and Beaker 4 should contain a concentration of NaCl of your own choosing. (Hint: a 1% solution of NaCl is 1 g of NaCl in 100 mL of liquid.)

DATA TABLE 1: NACI CONCENTRATIONS

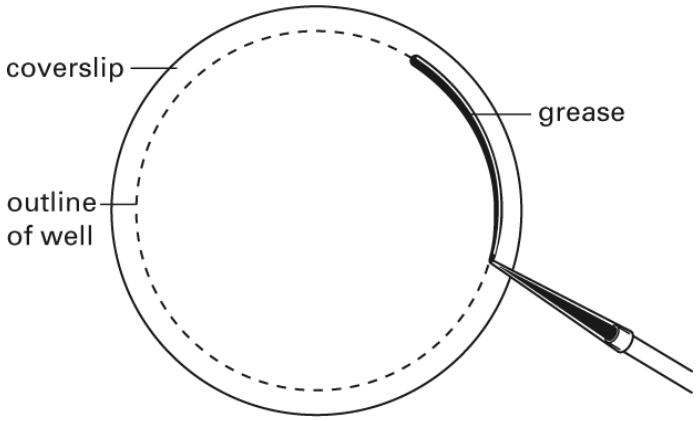
BEAKER	NACL CONCENTRATION (%)	AMOUNT OF NACL TO BE ADDED TO 5 ML (G)
1	2	
2	3	
3	5	
4		

Check with your teacher before continuing.

6. For each solution, weigh out the correct amount of NaCl.
7. Carefully add the salt to the appropriate beakers. Swirl the beaker while the salt is added, and do not add salt so fast that it accumulates on the bottom of the beaker.
8. Turn a 24-well plate upside down and label four of the wells 1–4.
9. Turn the 24-well plate right side up again, and place 1 mL of solution from Beaker 1 into Well 1, 1 mL of solution from Beaker 2 into Well 2, and so on. Use a new pipette for each solution.



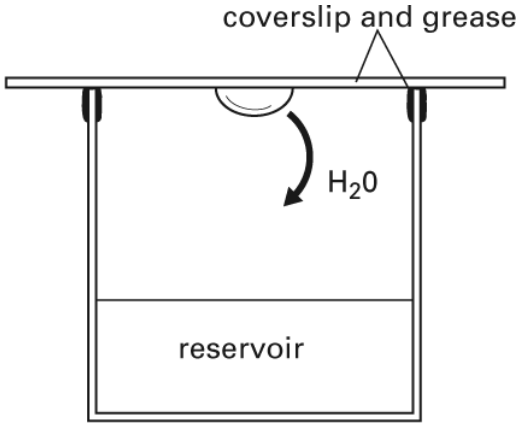
10. Get four round glass cover slips, and place them on top of the wells in the plate that are directly adjacent to the wells you filled.
11. Using the rim of the well as a guide, put a circle of grease onto the cover slip. Make sure that no grease touches the center of the cover slip.



12. Place a 2 μL drop of solution from Beaker 1 onto the center of the cover slip that is next to well 1, a 2 μL drop of solution from Beaker 2 onto the center of the cover slip that is next to Well 2, and so on. Use a new tip for each solution.

13. To each of these drops, add 2 μL lysozyme solution. Make sure the drops mix.

14. Invert cover slip 1 onto the plate so that the grease ring seals the cover slip over well 1 and the drop is hanging down from the cover slip. Repeat this with the other cover slips.



15. Put your plate in a safe place and leave it undisturbed.

16. Examine your samples every few days under the microscope. Note their appearance in Data Table 2. Draw any crystals you see in your Evidence Notebook.

DATA TABLE 2: CRYSTAL FORMATION

DAY AND TIME	NACL CONCENTRATION = 2%	NACL CONCENTRATION = 3%	NACL CONCENTRATION = 5%	NACL CONCENTRATION = _____ %

ANALYZE AND CONCLUDE

1. What was the purpose of using grease in the hanging drop method?

_____

_____

2. In which concentration(s) of salt did crystals form?

_____

_____

3. How was the crystals' appearance affected by salt?

_____

_____

4. Describe the appearance of each of your crystals under the microscope.

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5. How might a deletion in the lysozyme gene affect the structure of the protein?

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6. Would a point mutation or a frameshift mutation be more likely to alter the structure of a protein? Explain.

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