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Protein Degradation Study

Teacher's Guidebook

(Cat. # BE-412)

MATERIALS INCLUDED

This kit has enough materials and reagents for 24 students (six groups of four students).

- 1 vial Protein: Bovine Protein
- 1 vial Protease: Protease K
- 1 vial Protease-T Buffer
- 1 vial PAGE: Sample Loading Buffer
- 1 vial Stop Solution
- 90 Centrifuge Tubes (1.5ml)
- 1 bottle LabSafe GelBlue

SPECIAL HANDLING INSTRUCTIONS

- Store Protein: Bovine Protein and Protease K vials at 4°C.
- All other reagents can be stored at room temperature.
- Briefly centrifuge all small vials before opening to prevent waste of reagents.

ADDITIONAL EQUIPMENT REQUIRED

- Devices and reagents for making and running polyacrylamide gel electrophoresis.
- 8-12% SDS polyacrylamide electrophoresis gel.
- Stopwatch timer.

TIME REQUIRED

- 2-3 hours

OBJECTIVES

- Study how protein fragments degrade.
- Study the action of Protease K in the degradation of proteins into peptides.

BACKGROUND

A protein's life cycle begins with its initial synthesis and correct folding. The second part of the life cycle involves the control of the expression levels of a protein by regulating its degradation. The lifespan of proteins vary greatly and are dependent on several factors. For example, the half-lives of various enzymes in rat liver range from 12 minutes to 150 hours.

The "N-end" rule is the first factor to consider in the lifespan of a protein. Basically, the amino acid residue on the N-terminus of a protein can sometimes be used to predict the protein's lifespan. On average, proteins with an N-terminal Met, Ser, Ala, Thr, Val, or Gly have half lives greater than 20 hours and with N-terminal Phe, Leu, Asp, Lys, or Arg have half lives of 3 min or less.

The "PEST" factor is for proteins that are enriched in Pro (P), Glu (E), Ser (S) and Thr (T) and these are often degraded more rapidly than other proteins.

The most common degradative pathway for proteins is the ubiquitin-proteosome pathway. Proteins to be degraded are tagged with chains of ubiquitin, a monomeric 76 amino acid protein that becomes the signal that directs the protein to the proteasome. The proteasome is a multimeric protein that degrades proteins into short peptide sequences. Ubiquitin is attached to the "condemned" protein in an ATP dependent manner, using a series of 3 enzymes. Initially, the terminal carboxyl group of ubiquitin is joined in a thioester bond to a cysteine residue on Ubiquitin-Activating Enzyme (E1). This is the ATP-dependent step. The ubiquitin is then transferred to a sulfhydryl group on a Ubiquitin-Conjugating Enzyme (E2) and finally a Ubiquitin-Protein Ligase (E3) then transfers the activated ubiquitin to the ε-amino group of a lysine residue of a protein recognized by that E3, forming an isopeptide bond.

The proteasome is a large multimeric protein found in the cytosol and nucleus of a cell. In mammalian cells, the proteasome contains 2 copies of 14 different proteins that are arranged into 4 ring-like structures. The proteins to be degraded pass through the center of the proteasome where they are degraded. Specific cap proteins regulate protein entry to the proteasome.

This experiment uses protein electrophoresis to demonstrate the effects of proteases on a protein.

TEACHER'S PRE EXPERIMENT SET UP

Acrylamide/Bis-acrylamide is toxic. Always wear gloves and protective eyewear when handling the chemicals.

1. Prepare one 10-12% polyacrylamide gel containing 0.1% SDS (SDS-PAGE) or use premade electrophoresis gel with a 10 lane comb for each student group (G-Biosciences Protein Electrophoresis Kit (Cat. # BE-406) is recommended).

2. Prepare Electrophoresis Running Buffer contains 0.024M Tris base, 0.192M Glycine and 0.1% SDS.

3. Add 700µl Protease-T Buffer to Protein: Bovine Protein vial. Vortex the tube to dissolve the pellet completely. Aliquot 100µl to each student group.

4. Add 700µl Protease-T Buffer to Protease K vial. Vortex the tube to make uniform solution. Transfer 35µl to a new tube and add 665µl Protease T-Buffer to dilute the enzyme 1:20. Supply each group with 100µl.
   NOTE: *If performing the experiment with multiple groups, we recommend diluting the Protease K by different dilution factors. Recommend are a 1:5 dilution of the initial resuspended Protease K. This 1:5 dilution can be serially diluted 1:1 with buffer to give dilutions of 1:5, 1:10, 1:20, 1:40, 1:80 and 1:160. This will allow students to see the effect of protease amount on digestion.*
   i.e. Transfer 40µl of the Protease K after the initial vortex and add to 160µl Protease T-Buffer. Set up five tubes with 100µl Protease T-Buffer and label 1:10, 1:20, 1:40, 1:80 and 1:160. Pipette 100µl from the 1:5 dilution into the 1:10 tube and vortex. Repeat above but pipette from 1:10 tube to 1:20 tube. Continue until all dilutions have been made

5. Aliquot reagents for each student group according to the next section.

MATERIALS FOR EACH GROUP

Supply each group with the following components. Several components will be shared by the whole class and should be kept on a communal table.

- 1 10-12% Polyacrylamide Gel containing SDS
- 100µl Protein: Bovine Protein
- 100µl Protease K
- 120µl Stop Solution
- 330µl PAGE: Sample Loading Buffer
- 11 Centrifuge Tubes (1.5ml)
- Electrophoresis Running Buffer (shared with whole class)
- 50ml LabSafe GelBlue

PROCEDURE

Polyacrylamide gels contain Acrylamide/Bis-acrylamide that is toxic. Always wear gloves and protective device when handling the gel.

1. Label 10 tubes (1-10) and aliquot 10µl Protein:Bovine Protein to each tube.

2. Add 20µl Stop Solution to tube #10 and mix content by pipetting up and down 3-4 times.

3. Add 10µl Protease K to ***only*** the tube marked #1. Mix content by pipetting up and down 3-4 times. After mixing, start the timer.

4. Add 10µl Protease K to tubes 2-9 when the timer reaches the time points indicated in the table below.

5. Assemble the electrophoresis gel according to your teacher's instructions during the longer incubation times.

Tube#	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Bovine Protein	10µl	10µl	10µl	10µl	10µl	10µl	10µl	10µl	10µl	10µl
Timer (min)	0	15	30	40	50	55	58	59	60	----
Incubation time (min)	60	45	30	20	10	5	2	1	0	----

6. After adding Protease K to the tube 9, immediately add 10µl Stop Solution to tubes 1-9. Mix content by pipetting up and down 3-4 times.

7. Add 30µl PAGE: Sample Loading Buffer to each tube and load 20-30µl sample from each tube to the electrophoresis gel.

8. Start the electrophoresis run immediately after loading the last lane. Run the gel at 30mA/gel until the blue dye front has migrated to 0.5-2cm from the bottom.

9. Wash the gel twice in distilled water, five minutes each.

10. Remove all free water from the gel.

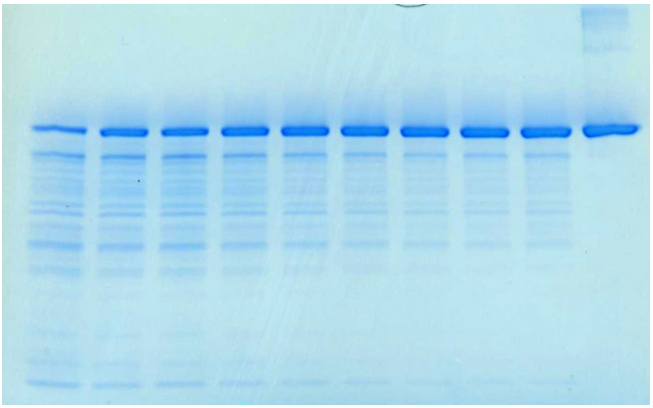
11. Add 50ml LabSafe GelBlue stain to cover the gel. Gently shake the gel for 60 minutes. Protein bands will start to appear after 5-10 minutes and can be readily viewed.

12. For optimal staining, decant the LabSafe GelBlue and rinse the gel with distilled water. The gel can be stored in water. Longer destain in water will give you a clearer view.

RESULTS, ANALYSIS & ASSESSMENT

Describe the appearance of the protein bands on the gel and discuss their significance with respect to degradation.

The fading of prominent protein band becomes visible as the incubation time with the protease is increased. This is due to the general protease having more time to digest the protein.



Q. Compare the role of the protease used in this experiment to the proteasomes and proteases in cells.

A. *The proteasome and proteases in cells breakdown large proteins into smaller fragments, or peptides, so that they can be removed from the cell. The protease in the experiment degraded a large protein into smaller peptides.*

Q. Why are cellular proteases stored in specific compartments within a cell?

A. *The experiment demonstrated that proteases rapidly degrade proteins. If proteases were allowed to move freely around a cell they will rapidly degrade functional proteins and as result inhibit a cell's function.*

Q. Describe two roles for protein degradation

A. *1. To degrade and remove damaged, misfolded or incorrectly synthesized proteins. 2. To rapidly remove proteins as a regulatory step.*

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