Bacterial Expression of Plant Pyruvate Kinase
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Glycolysis produces multiple important products. Formation of these products is in competition. Organisms produce ideal ratios for their own health and growth. Pyruvate kinase catalyzes the final step and so influences how much of each product forms. Amino acids vs carotenoids. Humans could make more nutritional (for us) food.
Objectives

- Originally
  - Express and purify two different isozymes of plant pyruvate kinase from *Arabidopsis thaliana*
  - Study their regulatory properties
    - Activators and inhibitors for mixed PK include glucose, insulin, glucagon
- Eventually
  - Express and purify two different isozymes of plant pyruvate kinase from *Arabidopsis thaliana*
  - Promote proper folding of the proteins after lysing
  - Maintain proper folding of the proteins after fractionating
  - Assess activity of the protein
1. Grow Cells:
Grow *E. coli* culture with gene for desired isozyme on agar plate, inoculate broth, incubate until desired optical density is reached

2. Isolate Cells:
Add IPTG to induce expression, incubate further, centrifuge and remove supernatant containing the broth

3. Isolate Cell Proteins:
Add lysozyme and incubate, sonicate, centrifuge and remove pellet containing cell membranes and unbroken cells

1.5 Ice Bath:
30 or 60 minute ice bath for experimental groups

4. Separate Different Proteins:
Use chromatography to separate supernatant into fractions, collect fractions (~20), add arginine and glycerol to half of each fraction

5a. Locate Proteins:
Use Bradford’s Reagent to check each fraction for protein, fraction with most protein (brightest blue) will be used for tests in 5b and 5c

5b. Check Activity of Proteins:
Run assay in spectrophotometer to check for oxidation of NADH after addition of choice fraction

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5c. Verify Protein Identity:
Run protein electrophoresis on choice fraction and neighbors to check molecular weight of protein

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Making Pyruvate Kinase

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2. Isolate Cells: Add IPTG to induce gene expression, incubate further, centrifuge and remove supernatant containing the broth
1. **Grow Cells:**
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Finding Pyruvate Kinase

3. Isolate Cell Proteins: Add lysozyme, sonicate, centrifuge, remove pellet containing cell membranes and unbroken cells.

4. Separate Different Proteins: Use chromatography to separate supernatant into fractions, collect fractions (~20), add stabilizer to half of each fraction.

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1. Grow Cells:
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Run protein electrophoresis on choice fraction and neighbors to check molecular weight of protein.
Checking Results

- 5c. Verify Protein Identity: Run protein electrophoresis on choice fraction and neighbors to check molecular weight of protein

- 5b. Check Activity of Proteins: Run assay in spectrophotometer to check for activity of choice fraction
Results

• For trial with first isozyme, found protein was not isolated and protein precipitated out rapidly succeeding fractionating
• For second isozyme, protein remained soluble
Discussion

• Stabilizer does help keep pyruvate kinase folded, stabilized results did not scatter the light in the assay
• Producing isolated pyruvate kinase is insufficient
  • Another component is needed, other molecules found in the same area of Arabidopsis as this isozyme of PK should be added for activity
• Ice bath does increases concentration of soluble protein

Quantitative Bradford’s Assay of Pyruvate Kinase

0 min total= 1.5964mg PK
60 min total= 4.7057mg PK
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Questions?