Bacterial Expression of Plant Pyruvate Kinase

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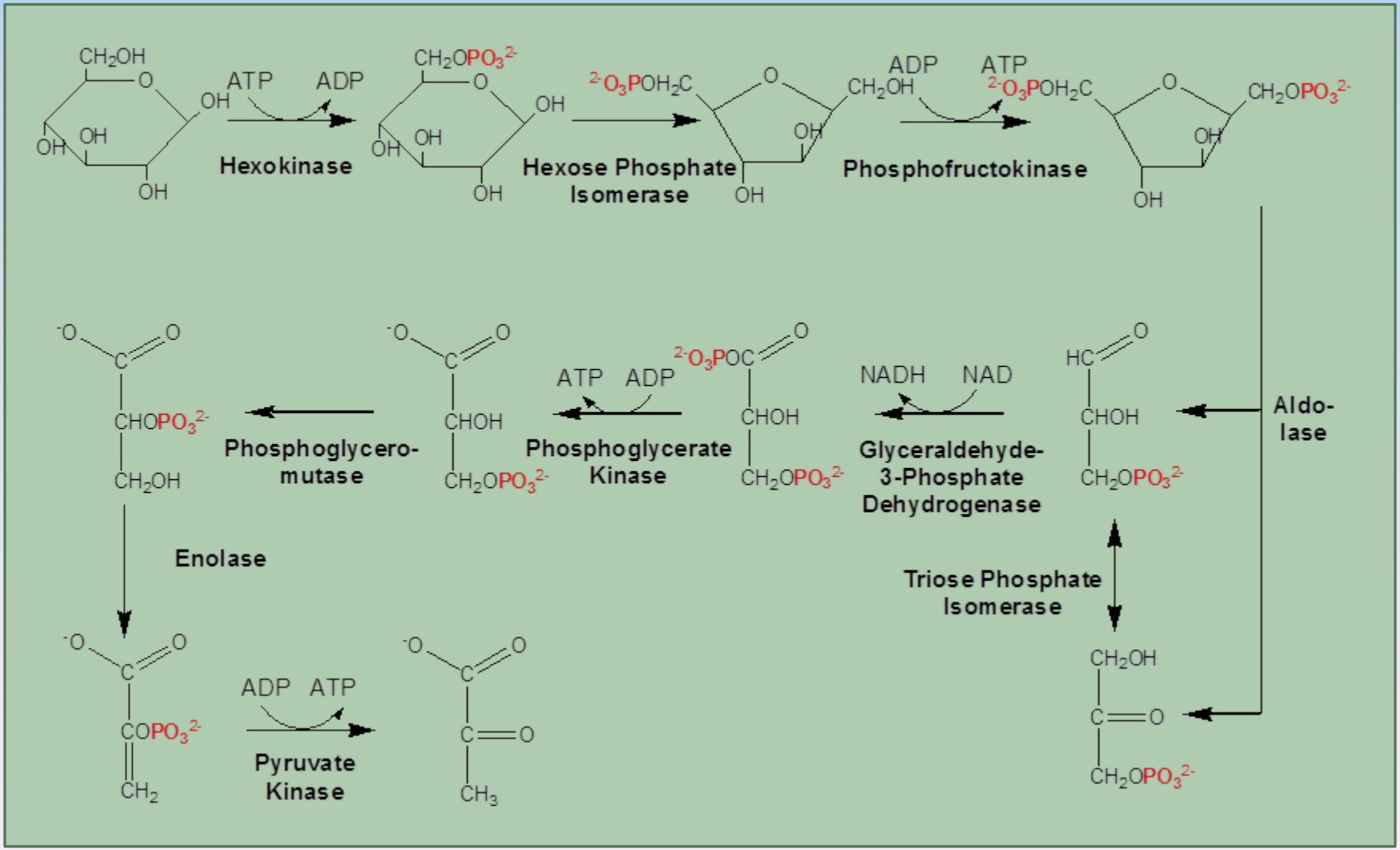
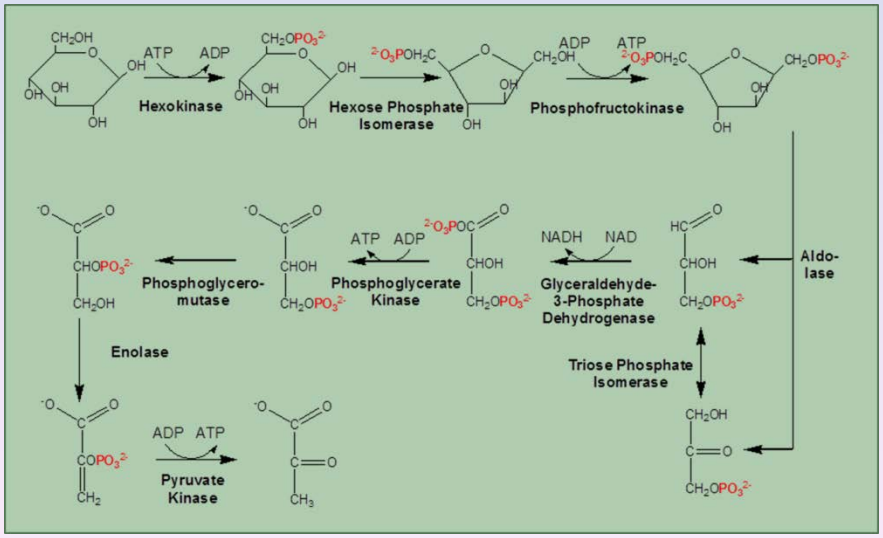
Background


ATP
ADP
Hexokinase
Hexose Phosphate Isomerase
ADP
ATP
Phosphofructokinase
Aldo-lase
NADH
NAD
Glyceraldehyde-3-Phosphate Dehydrogenase
ATP
ADP
Phosphoglycerate Kinase
Phosphoglycero-mutase
Enolase
Triose Phosphate Isomerase
ADP
ATP
Pyruvate Kinase

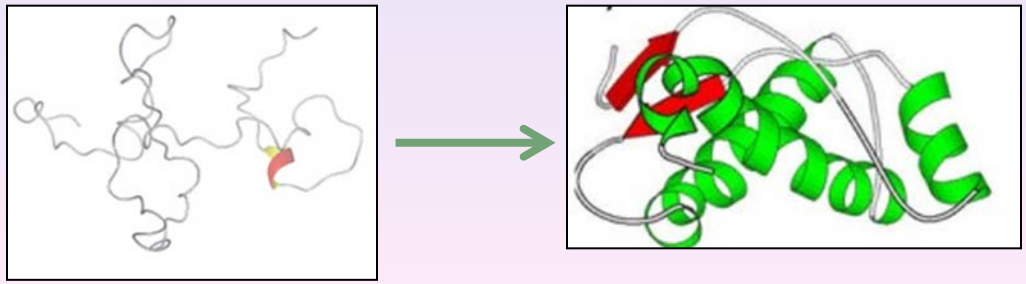
Background

- 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



Methods

1. Grow Cells:

Grow *E. coli* culture with gene for desired isozyme on agar plate, inoculate broth, incubate until desired optical density is reached

1.5 Ice Bath:

30 or 60 minute ice bath for experimental groups

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

4. Separate Different Proteins:

Use chromotography to separate supernatant into fractions, collect fractions (~20), add arginine and glycerol to half of each fraction

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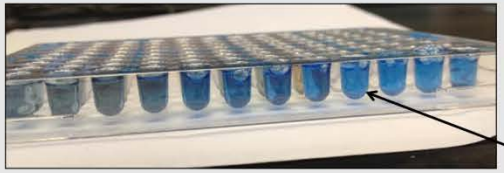

5a. Locate Proteins:

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

4 4

5b. Check Activity of Proteins:

Run assay in spectrophotometer to check for oxidation of NADH after addition of choice fraction

5c. Verify Protein Identity:

Run protein electrophoresis on choice fraction and neighbors to check molecular weight of protein

Making Pyruvate Kinase

- 1. Grow Cells: Grow *E. coli* culture with gene for desired isozyme on agar plate, inoculate broth, incubate until desired optical density is reached
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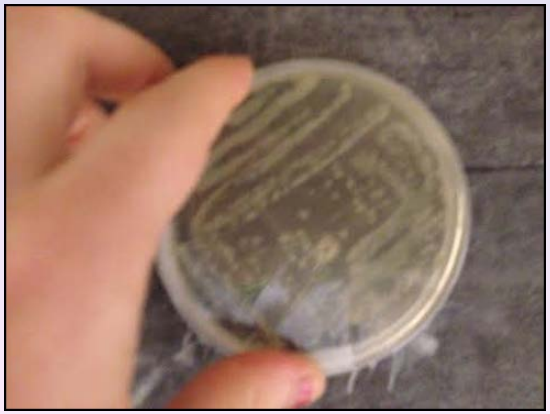

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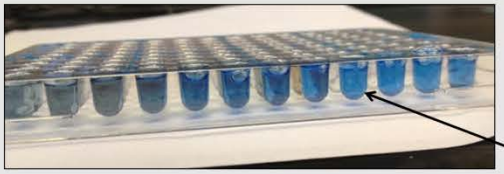

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Finding Pyruvate Kinase

- 3. Isolate Cell Proteins: Add lysozyme, sonicate, centrifuge, remove pellet containing cell membranes and unbroken cells
- 4. Separate Different Proteins: Use chromotography to separate supernatant into fractions, collect fractions (~20), add stabilizer to half of each fraction
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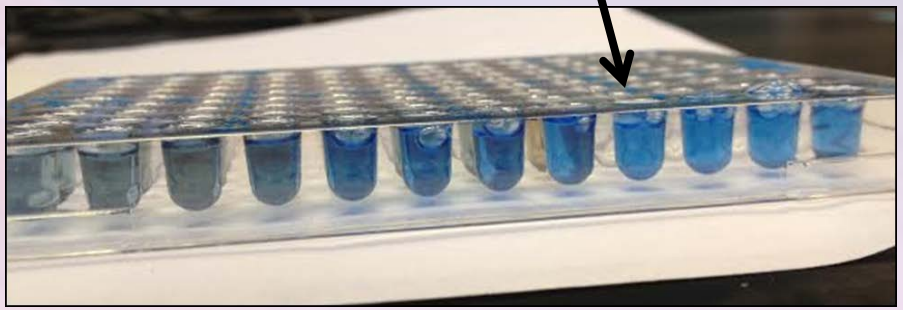

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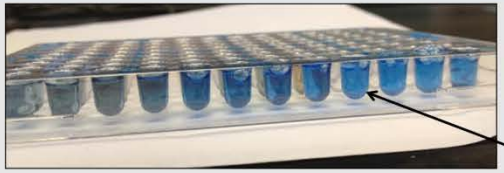

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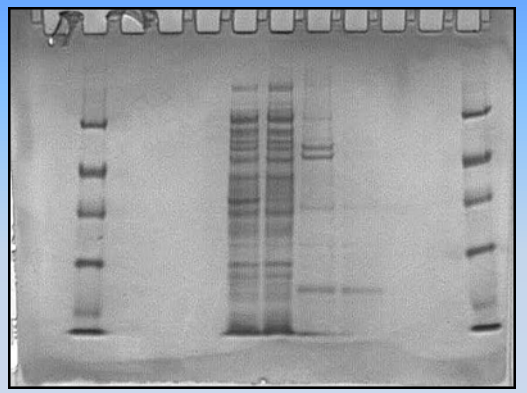
Checking Results



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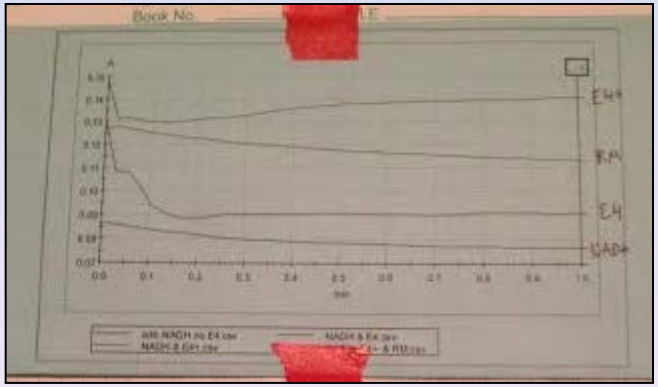
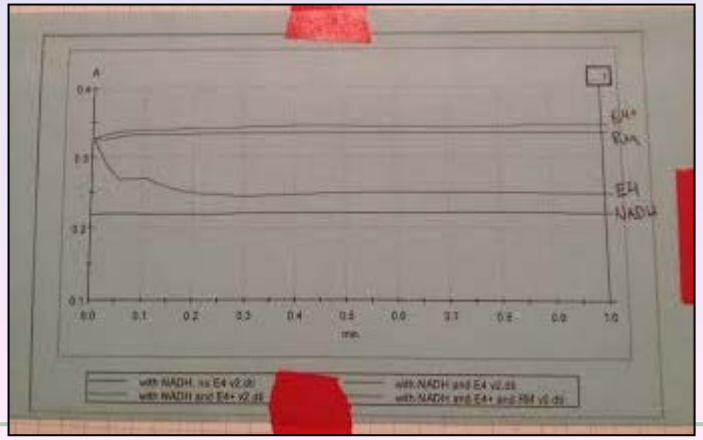
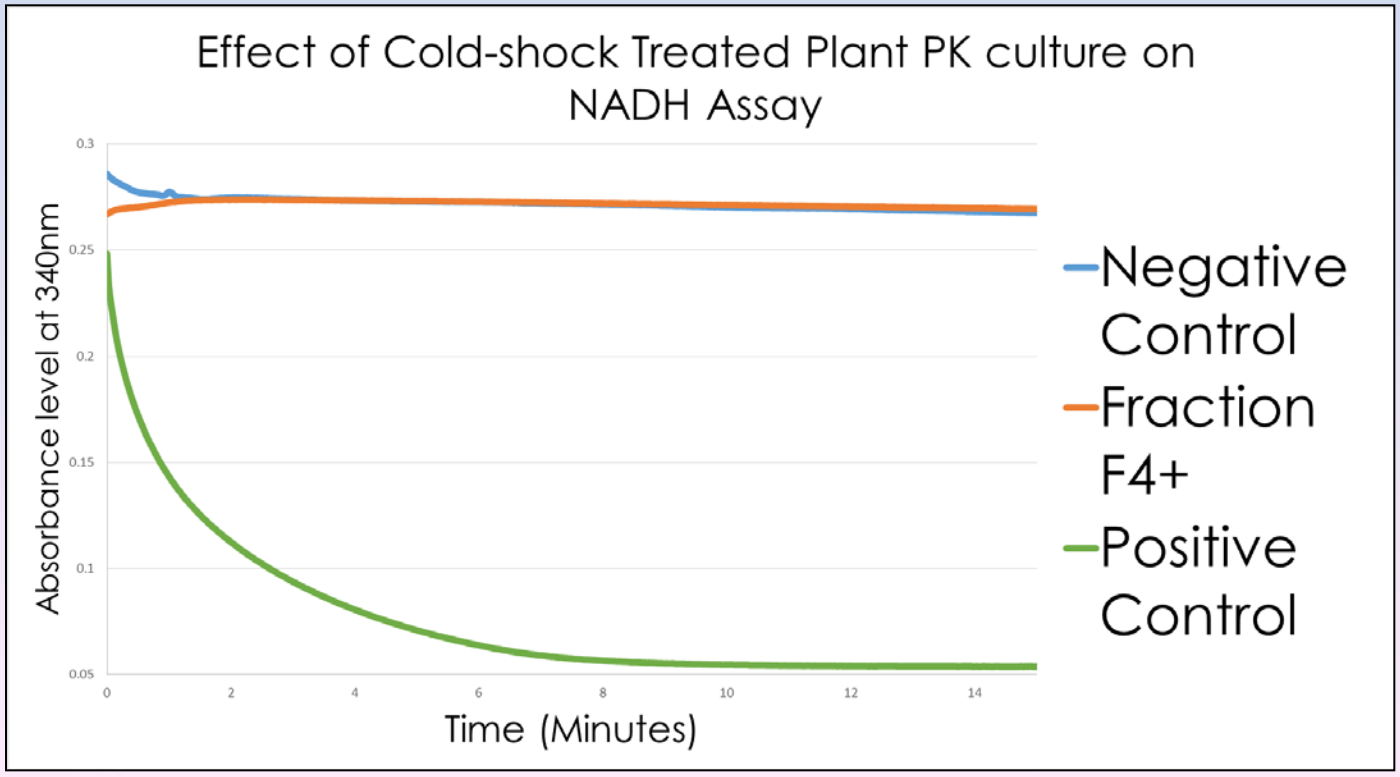




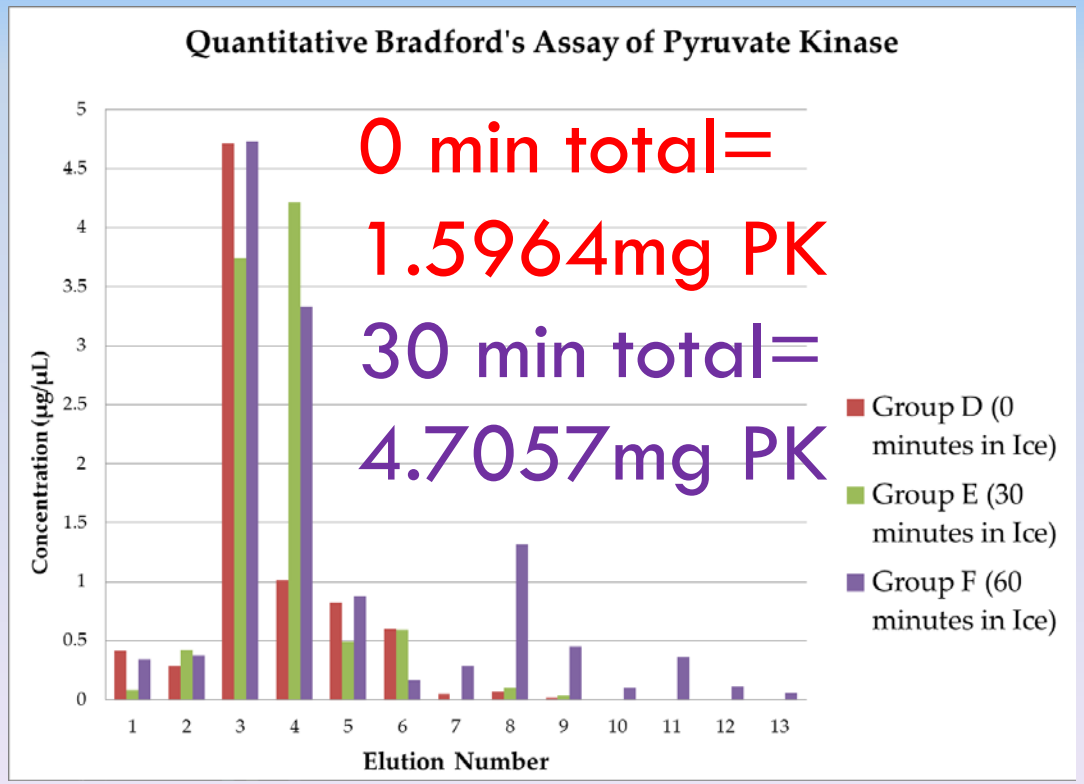
Results

- 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

Acknowledgements

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Questions?

http://suddenlyseptember.com/wp-content/uploads/2015/02/Ask-The-Right-Questions-.jpg