Using alkaline phosphatase extracted from bean beetles to introduce enzyme characteristics in introductory biology

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Why use this system?

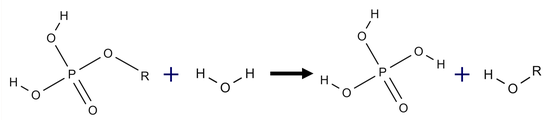
- There is interest in understanding phosphatases
  - Involved in regulation of enzymes and receptors
  - Dysregulation linked to various diseases and disorders (Tonks 2006, Goebel-Goody et al. 2012, Lambroso et al. 2016)
- Can be used as a model for an introduction to enzymes, enzyme kinetics, the role of cofactors, chirality of molecules, and effects of inhibitors on enzyme activity
- Bovine intestinal alkaline phosphatases are <40% similar in sequence to bean beetle alkaline phosphatase (protein BLAST using uniprot.org)
- Can compare kinetics and regulation of activity

What should students know?

- Basic structure of proteins
- Basics about enzymes
- The basics of regulation of enzymatic activity

Enzyme assay

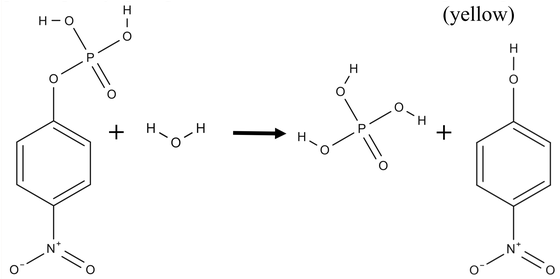
Typical reaction catalyzed by alkaline phosphatase:



Reaction carried out by alkaline phosphatase in the presence of the synthetic substrate p-nitrophenyl phosphate:

p-nitrophenyl phosphate

p-nitrophenol (yellow)



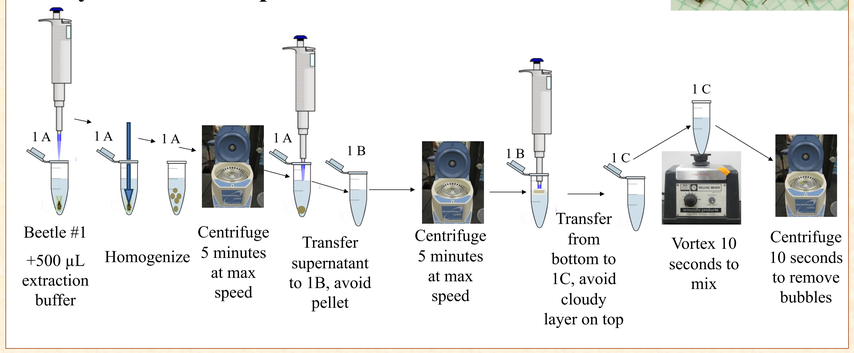
The substrate p-nitrophenyl phosphate is hydrolyzed to produce the products p-nitrophenol (yellow) and inorganic phosphate.

Materials

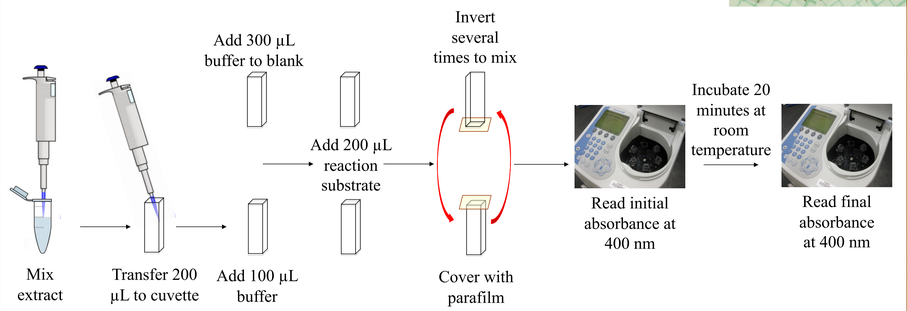
- Bean beetles, *Callosobruchus maculatus*
- Incubator with light and temperature control
- Extraction buffer (Tris-HCl, 50 mM, pH 8.5, 1% Triton X v/v) with and without 5 mM $MgCl_2$
- Buffer (Tris-HCl, 50 mM, pH 8.5) with and without $MgCl_2$
- p-nitrophenyl phosphate (20 mM in buffer) with and without $MgCl_2$
- L- and D- Phenylalanine (20 mM in buffer) with $MgCl_2$
- Bradford reagent
- BSA for a standard curve
- Spectrophotometer, vortexer, centrifuge
- Other standard laboratory equipment

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Enzyme extraction procedure



Standard enzyme assay procedure



Sample results

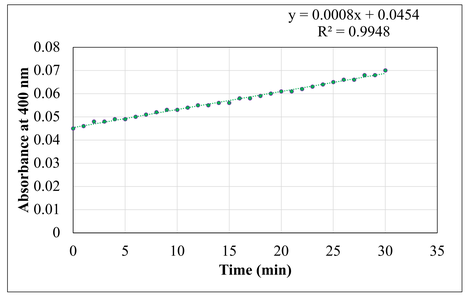


Figure 1: Change in absorbance over time for the enzyme reaction for enzyme extracted from one beetle.

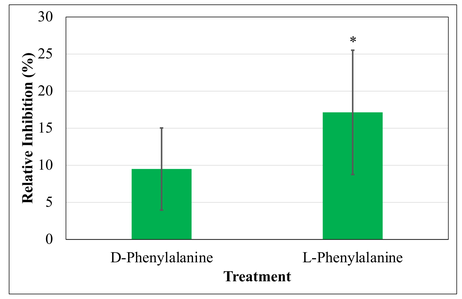


Figure 2: The effect of D- and L- Phenylalanine on the rate of reaction of alkaline phosphatase extracted from bean beetles. Columns represent means and error bars represent SEM. N = 10 per treatment.
* denotes statistically significant effect at $p < 0.05$.

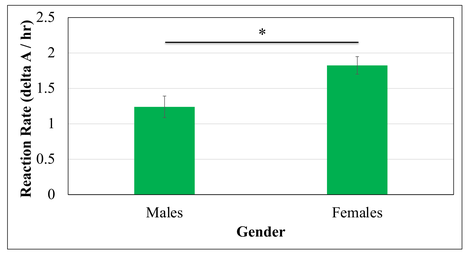


Figure 3: Difference in rate of reaction of alkaline phosphatase between the sexes. Columns represent means and error bars represent SEM. N = 5 per treatment.
* denotes statistically significant effect at $p < 0.05$.

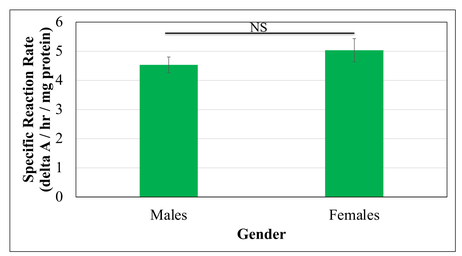


Figure 4: Difference in rate of reaction of alkaline phosphatase between the sexes, when corrected for protein content. Columns represent means and error bars represent SEM. N = 5 per treatment.

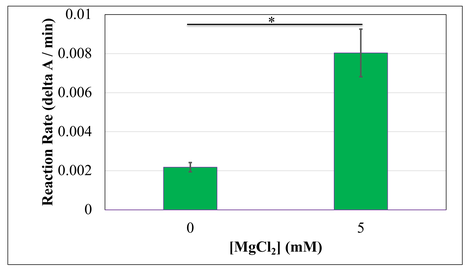


Figure 5: Difference in rate of reaction of alkaline phosphatase with and without $MgCl_2$ for each beetle (paired). Columns represent means and error bars represent SEM. N = 10.
* denotes statistically significant effect at $p < 0.05$.

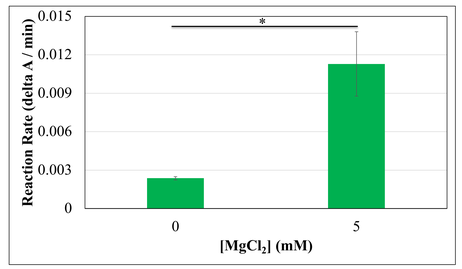


Figure 6: Difference in rate of reaction of alkaline phosphatase with and without $MgCl_2$ for different beetles (unpaired). Columns represent means and error bars represent SEM. N = 5 per treatment.
* denotes statistically significant effect at $p < 0.05$.

Comments

- Easily done with introductory students
- Can be done in regular 3-hour laboratories
- The topic can be expanded to include:
  - Enzyme kinetics: Km and Vmax with cofactors and inhibitors
  - Bioinformatics: comparison with commercially available intestinal bovine alkaline phosphatase, or other insect enzymes reported in the literature

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References

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