Characterization of Invertase from *Saccharomyces cerevisiae*

Experiment Outline

Temperature sensitivity ✔ *Done in Lab 7*

- Determine which temperatures the enzyme works best at.
- Probably related to the optimal living temperature for the yeast.

pH optimum ✔ *Done in Lab 7*

- Determine which pHs the enzyme works best at.

Enzyme kinetics ✔ *Done in Lab 7*

- Determine activity of enzyme at different concentrations of sucrose.
- Amount of product formed allows for production of Lineweaver-Burk plot
  - Determines K_m.
    - The affinity of the enzyme for the substrate.
  - Determines V_{max}.
    - Maximal velocity of enzyme.
    - How fast the enzyme works when saturated with substrate.

Size of Enzyme (with SDS-PAGE) ❑ *To-Do*

- Determine molecular weight of enzyme in kD
  - Run a lane of molecular weight standards on gel
    - Myosin (200 kD), β galactosidase (116.3 kD), Phosphorylase b (97.4 kD), BSA (66.3 kD), Glutamic dehydrogenase (55.4 kD), Lactate dehydrogenase (36.5 kD), Carbonic anhydrase (31 kD), Trypsin inhibitor (21.5 kD), Lysozyme (14.4 kD)
    - Invertase should be around 150 kD.
      - So, band for it should be between Myosin and β galactosidase.
        - That assumes invertase is one only subunit!
  - Run a second lane with invertase.
- SDS-PAGE also determines number of subunits in the enzyme.
  - If there is more than one subunit, then more than one band will appear.
    - Then molecular weight is the sum of these subunits.
  - Reducing agents such as mercaptoethanol or DTT (dithiothreitol) are used to break disulfide bonds that could be covalently linking two subunits within the protein.
  - We can then characterize if subunits exist, and if they do, if they are covalently linked with disulfide bonds (which is common for extracellular proteins).
  - It would be surprising to find that invertase had subunits linked together with disulfide bonds. We are presumably using an intracellular invertase (although there is an extracellular invertase), and the interior environment of the cell is highly reducing.

pI of Enzyme ❑ *To-Do*

- ❑ *To-Do*: Figure out what the pI of an enzyme tells us.
- Two methods to try.
- Agarose Gel Electrophoresis.
  - We did this in Lab 7.
  - Run lanes of standards.
  - Run one lane with invertase.
  - Figure out pI based on how far it traveled in comparison to standards
- Isoelectric focusing.
  - ❑ *To-Do:* research how to perform this.
    - It doesn't seem hard to do, from the book's description.
  - Use known standards with invertase.
    - Standards will "focus" on their known pI values.
  - The pI of the unknown (invertase) can be determined based on positions of standards.
- Compare the results of the two methods for the poster.
  - Isoelectric focusing is likely to be more accurate.

Activity of Enzyme ❑ *To-Do*

- It is known that invertase reacts with sucrose.
  - Sucrose = glucose + fructose.
  - What other polysaccharides can invertase react with besides sucrose?
  - Two classes to investigate, since sucrose is composed one of each class.
    - α-glucosides:
      - Maltose, Cellobiose, Trehalose – all reducing sugars.
    - β-fructofuranosides:
      - Raffinose – an unsubstituted β-fructofuranoside; non-reducing sugar.
      - Melezitose – a substituted β-fructofuranoside; non-reducing sugar.
        - Means that there is something attached to the fructose group.
- Invertase is likely to look for sugars with a terminal glucose unit (α–glucoside) or a terminal fructose unit (β-fructofuranoside).
  - If it works best with β-fructofuranosides, then we can characterize invertase as a β-fructofuranosidase.
  - If it works best with α-glucosides, then we can characterize invertase as a α-glucosidase.

Stability of Enzyme ❑ *To-Do*

- Enzymes are sensitive to their environment (pH, temperature).
- Invertase may perform suboptimal (or not at all) if not prepared correctly.
- Test different preparations of invertase with identical samples of sucrose.
  - Dissolved with cool pH 4.5 buffer, and stored on ice (denoted "CI4").
    - Hypothesized to be the optimal preparation method.
  - Dissolved with room temp pH 4.5 buffer, and stored on ice ("RI4").
    - Dissolved in "warm" solvent may damage the enzyme.
  - Dissolved with cool pH 7 water, and stored on ice ("CI7").
    - The wrong pH may damage the enzyme.
  - Dissolved with cool pH 4.5 buffer, but left at room temp ("CR4").
    - The enzyme may be damaged as the room temperature heats the tube.
  - Dissolved with room temp pH 7 water, and left at room temp ("RR7").
    - The wrong pH may damage the enzyme.
    - The enzyme may be damaged as the room temperature heats the tube.
    - Hypothesized to be the worst preparation method.

Inhibition of Enzyme ❑ *To-Do*

- It is known that invertase is inhibited by harmaline. ✔ *This step done in Lab 7*
- Try other inhibitors and plot results on Lineweaver-Burk plots.
  - Fructose (tricky, because it is also a reducing sugar)
  - Sucralose (*Spenda*™ artificial sweetener)!
  - Palatinose (too expensive)?
  - Turanose (stockroom did not have it)