Emilia - Lab 4 Enzymes

Theory

Metabolic reactions overview
- Reactions that need energy to proceed = endergonic
  - Called non-spontaneous reactions
  - Reactant energy < Product energy (Memory tip: direction of inequality points in direction of the rxn)
- Reactions that release energy to proceed = exergonic
  - Called spontaneous reactions
  - Reactant energy > Product energy
- Activation energy (Ea) is the energy that must be absorbed by the reactants in order to proceed
  - Lecture notes example: The reaction of wood burning is exergonic since heat energy is released and products end up with less energy than the reactants. Some amount of energy is required however to get the reaction started (i.e. heat in the form of a match).
- Catalysts partially determine the rate of the reaction since they lower Ea.

Enzyme overview
- Enzymes are a type of biological catalysts because they speed up biological reactions
- They do NOT determine the direction of the reaction or the reactions final equilibrium
- Not used up in the reaction process
- Usually very substrate specific, thus allowing for precise control over individual metabolic pathways.
- Usually proteins

Binding terminology
- Substrates bind to the Active Site on an enzyme
- Bound enzyme + substrate called Enzyme Substrate Complex

Factors that affect enzyme activity and rates of reaction (Memory tip: “put a CAP on IT”)
- Concentration: Increasing enzyme or substrate will increase rate of reaction
- Activators: chemicals that must bind for the enzyme to be active
  - Cofactors: non-proteins that bind to the active site. Can be metal ions.
  - Coenzymes: organic cofactors
- pH: Too high/low can affect protein intermolecular interactions
- Inhibitors
  - Competitive inhibitors
    - Substances that bind to the active site on an enzyme
    - Adding additional substrate will reverse the inhibition. (Think of this in terms of probability: The competitive inhibitor will not bind forever and so when it drops off of the active site, if additional substrate has been added, chances are that the substrate will bind instead.
  - Non-competitive inhibitors
    - Substances that bind to a part of the enzyme that is NOT the active site
    - The inhibitor can become unbound, thus reversing the inhibition, however adding additional substrate will NOT reverse the inhibition
- Temperature: Too high/low can affect protein intermolecular interactions
  - Increased temp speeds up the motion of molecules → increased rate of reaction
  - Generally, an increase of 10°C = 2x-3x increase in rate of reaction (when near room temp)
  - Optimal temp vary by enzyme and depend on tertiary and quaternary structures
  - Higher temp → enzymes denature (non-reversible)
  - Lower temp → slow enzyme activity (reversible)

Lab experiments: measuring enzymes activity

4.1 Measuring Catechol Oxidase Activity
- Reaction: catechol + O2 → benzoquinone + H2O
- In the experiment, the potato extract contains the enzyme (Catechol Oxidase).
- Benzoquinone is the reason why fruits and vegetables turn brown in presence of O2. Thought to be antibacterial (quiz 4 extra credit question).
- Question: Will solution of catechol turn brown if the potato extract (enzyme) is added?
- Hypothesis: Solution of catechol will turn brown once potato extract is added.
- Prediction: If the hypothesis is true, then the tube #2 will turn brown since it is the only once where the enzyme (potato extract) is added to the substrate (catechol) and no reaction will be present in tubes 1 and 3.
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- Procedure and results

<table>
<thead>
<tr>
<th>Tube</th>
<th>Substrate</th>
<th>Enzyme</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water</td>
<td>Catechol</td>
<td>--- Clear, light beige color</td>
</tr>
<tr>
<td>2</td>
<td>Water</td>
<td>Catechol</td>
<td>Potato extract Instantly turned brown</td>
</tr>
<tr>
<td>3</td>
<td>Water</td>
<td>---</td>
<td>Potato extract Cloudy beige</td>
</tr>
</tbody>
</table>

- Experiment notes:
  - Controls: tubes 1, 3. Purpose of having two control tubes is to have a comparison for each of the components. Comparing tube 2 to 1 and 3 shows that a reaction is actually taking place.
  - The additional water is needed to keep the volume constant. Constant volume is needed, as this is a controlled variable.

4.2 Inhibition of catechol oxidase

- PTU (phenylthiourea) = inhibitor of catechol oxidase
- We know that PTU is a competitive inhibitor because the addition of more substrate (catechol) yielded product (brown colored solution).

  - Question/Hypothesis: Is PTU a competitive inhibitor? / PTU is a competitive inhibitor
  - Prediction: If true the hypothesis is true, then we expect product (brown) to be formed when additional substrate is added.

- Procedure and results

<table>
<thead>
<tr>
<th>Tube</th>
<th>DI</th>
<th>Enzyme</th>
<th>PTU</th>
<th>DI</th>
<th>Substrate</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5ml</td>
<td>0.5ml</td>
<td>0.5ml</td>
<td>0.5ml</td>
<td>0.5ml</td>
<td>Little change</td>
</tr>
<tr>
<td>2</td>
<td>5ml</td>
<td>0.5ml</td>
<td>0.5ml</td>
<td>---</td>
<td>1ml</td>
<td>Brown =&gt; enzyme working</td>
</tr>
<tr>
<td>3</td>
<td>5ml</td>
<td>0.5ml</td>
<td>---</td>
<td>1ml</td>
<td>0.5ml</td>
<td>Brown</td>
</tr>
</tbody>
</table>

- Experiment notes:
  - Control: tube 3
  - Concentration of catechol (substrate) increased in tube 2 to demonstrate that competitive inhibition is reversible by adding additional substrate.
  - Catechol was added last so that it wouldn’t have a chance to react with the enzyme before PTU was added.

4.3 Concentration, pH, and temperature influence on Amylase activity

- Amylase is an enzyme that breaks down amylase in starch \( \rightarrow \) maltose
- Amylase is produced in salivary glands and pancreas and is responsible for preliminary digestion (quiz 4)
- Testing for amylase activity: test for disappearance of substrate (NOT production of product as in 4.1)
  - Add iodine to solution:
    - If starch present \( \rightarrow \) dark purple (amylase in starch combines with iodine)
    - If no starch (meaning that amylase has converted starch to maltose) \( \rightarrow \) NO color change (stays dark amber/yellow)
- Graphing in exp. A-C: remember that y-axis (time) goes from “slow” (i.e. 5 mins) to “fast” (i.e. 1.5 mins)

4.3 A: Influence of enzyme concentration on rate of starch digestion by amylase to maltose

- Conclusion: INCREASING amylase enzyme concentration INCREASES reaction rate
- Independent variable: amylase concentration
- Dependent variable: time of starch disappearance
- Graph of amylase concentration vs. time of starch disappearance: LINEAR, positive slope

4.3 B: Influence of pH on rate of starch digestion by amylase to maltose

- Conclusion: pH 7 is the optimal pH for amylase
- Independent variable: pH
- Dependent variable: time of starch disappearance
- Graph of pH vs. time of starch disappearance: BELL shape (at pH4 and pH9 time is “slow”, while at pH7 time is “fast”)

4.3 C: Influence of temperature on rate of starch digestion by amylase to maltose

- Conclusion: INCREASE temperature INCREASES rate of reaction…BUT if too high \( \rightarrow \) enzyme denatures
- Independent variable: temperature
- Dependent variable: time of starch disappearance
- Graph of temperature vs. time of starch disappearance: BELL shape (rate at low and high temp is “slow”, while at 37C, reaction rate is “fast”)

Applying your knowledge
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1. Evidence of catechol oxidase provided by brown color of solution
2. This question is about pH. Fresh pineapple has a different acidity (pH) than canned, which prevents the enzyme in the gelatin from working. (**Quiz 4 question 1**)
3. This is about optimal temperatures. Cold temperatures greatly reduced the enzymatic activity.
4. Ethanol is a competitive inhibitor for the enzyme that converts ethylene glycol to its 4 toxic products. Ethanol is administered intravenously for hours in order to maintain the competitive block. (**Quiz 4 question 7**)
5. Graph A shows a fast reaction rate at low temperatures and a slow reaction rate at high temperatures and is therefore the best representation of thermolabile enzyme activity.