Assay of Amylase from saliva

Aim:

To assay the activity of amylase enzyme present in saliva.

Principle:

Amylase is an enzyme that catalyzes the hydrolysis of starch into simpler sugars such as maltose and glucose. The assay measures the rate at which amylase breaks down starch, typically using iodine as an indicator that changes color from blue-black (starch present) to reddish-brown (starch absent).

Materials and Methodology:

Materials:

- Saliva samples
- Starch solution
- Iodine solution
- Buffer solution (e.g., phosphate buffer)
- Test tubes
- Pipettes
- Water bath
- Spectrophotometer

Methodology:

1. **Preparation:**
    - Prepare a series of test tubes labeled accordingly.
    - Prepare starch solution and buffer solution.
    - Set up water baths at appropriate temperatures.
2. **Procedure:**
    - **Step 1: Preparation of Reaction Mixtures**
        - In each test tube, mix varying amounts of saliva (diluted appropriately) with starch solution and buffer.
        - Start with a control tube containing only starch and buffer (no saliva).
    - **Step 2: Incubation**
        - Incubate all tubes at a specified temperature (usually around body temperature, ~37°C) for a fixed time period.
    - **Step 3: Stopping the Reaction**
        - Add iodine solution to each tube to stop the enzymatic reaction after the incubation period.
    - **Step 4: Measurement**
        - Measure the absorbance of each tube at a specific wavelength (e.g., 540 nm) using a spectrophotometer.

- Higher absorbance indicates more remaining starch, implying lower amylase activity.

Step 5: Calculation

- Calculate the enzyme activity using the absorbance values and standard curves or known concentrations.

Given Result for Experiment:

Assume a hypothetical set of results where the absorbance values decrease as the concentration of saliva (and thus amylase activity) increases:

Saliva Dilution	**Absorbance at 540 nm**
0 (control)	High
1:2	Moderate
1:4	Low
1:8	Very Low

These results suggest that higher dilutions of saliva correspond to lower amylase activity, as evidenced by lower remaining starch (lower absorbance).

Assay of Acid phosphates from potato

Aim:

To quantify the activity of acid phosphatase in potato tissue.

Principle:

Acid phosphatase catalyzes the hydrolysis of phosphate esters under acidic conditions. The enzymatic activity can be measured by monitoring the release of phosphate using a colorimetric assay.

Materials and Methodology:

Materials:

- Potatoes
- Buffer solutions (e.g., sodium acetate buffer)
- Substrate solution (e.g., p-nitrophenyl phosphate)
- Stop solution (e.g., sodium hydroxide)
- Colorimetric reagent (e.g., ammonium molybdate)
- Spectrophotometer
- Cuvettes
- Water bath or incubator
- Pipettes and glassware

Methodology:

1. **Preparation of Extract:**
    - Grind fresh potato tissue in a chilled mortar and pestle with sodium acetate buffer (pH ~5.0).
    - Centrifuge the homogenate at a low temperature to obtain a clear supernatant (enzyme extract).
2. **Enzyme Assay:**
    - Prepare a reaction mixture containing the enzyme extract, substrate (p-nitrophenyl phosphate), and buffer.
    - Incubate the reaction mixture at a specific temperature (typically around 37°C) for a defined period (e.g., 30 minutes).
3. **Color Development:**
    - Stop the reaction by adding a stop solution (e.g., sodium hydroxide) to the reaction mixture.
    - Add a colorimetric reagent (e.g., ammonium molybdate) to develop a colored complex.
4. **Measurement:**
    - Measure the absorbance of the colored complex at a specific wavelength (typically around 405 nm) using a spectrophotometer.

Procedure:

1. **Preparation of Potato Extract:**
    - Peel and finely chop potatoes.
    - Grind the potato pieces with sodium acetate buffer (pH 5.0) in a mortar and pestle.
    - Centrifuge the homogenate at 10,000 rpm for 10 minutes at 4°C.
    - Collect the supernatant (enzyme extract) and keep it chilled.
2. **Enzyme Assay:**
    - Prepare a reaction mixture containing:
        - 0.5 mL of enzyme extract
        - 2.0 mL of sodium acetate buffer (pH 5.0)
        - 1.0 mL of 10 mM p-nitrophenyl phosphate substrate solution
    - Incubate the reaction mixture in a water bath at 37°C for 30 minutes.
3. **Color Development and Measurement:**
    - After incubation, add 3.0 mL of 0.1 M sodium hydroxide to stop the reaction.
    - Add 1.0 mL of 0.5 M ammonium molybdate solution to the reaction mixture and mix thoroughly.
    - Incubate the reaction mixture for 10 minutes at room temperature to develop the color.
    - Measure the absorbance of the solution at 405 nm using a spectrophotometer.

Given Result (Example):

- Absorbance (A): 0.550

Effect of substrate concentration on enzyme activity

Aim:

To investigate the effect of substrate concentration on the activity of the enzyme [enzyme name].

Principle:

Enzymes catalyze biochemical reactions, and their activity is influenced by various factors including substrate concentration. According to the Michaelis-Menten kinetics, enzyme activity initially increases with substrate concentration until it reaches a maximum rate (Vmax), where all enzyme active sites are saturated with substrate.

Materials and Methodology:

Materials:

- [List of materials]

Methodology:

1. **Preparation of enzyme solution:**
    - Prepare a stock solution of the enzyme.
    - Dilute the stock solution to obtain the desired enzyme concentration.
2. **Preparation of substrate solutions:**
    - Prepare a series of substrate solutions with varying concentrations (e.g., 1 mM, 2 mM, 3 mM, 4 mM, 5 mM).
3. **Experimental setup:**
    - Label test tubes or wells for each substrate concentration.
    - Prepare a control without substrate for baseline measurement.
4. **Procedure:**
    - Mix appropriate volumes of enzyme solution and substrate solution in each test tube or well.
    - Start a timer and incubate the reactions at a specified temperature for a fixed duration.
5. **Measurement of enzyme activity:**
    - After incubation, stop the reactions by adding a suitable stop solution.
    - Measure the product formed (e.g., using spectrophotometry, colorimetry, or another appropriate method) to determine enzyme activity.
    - Record absorbance or other relevant measurements.
6. **Data analysis:**
    - Plot a graph of enzyme activity (reaction rate) against substrate concentration.
    - Analyze the graph to determine parameters such as Vmax and Km (Michaelis constant) if applicable.

Expected Results:

- As substrate concentration increases, enzyme activity (reaction rate) initially increases.
- A point will be reached where further increases in substrate concentration do not significantly increase enzyme activity (enzyme saturation).

Example Graph:

java
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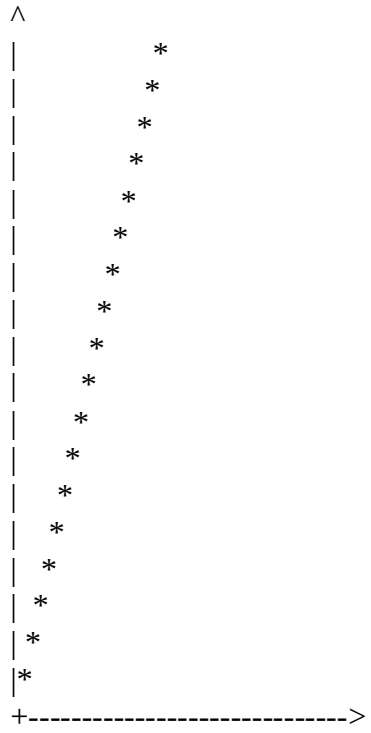

In the graph:

- **X-axis:** Substrate concentration (mM)
- **Y-axis:** Enzyme activity (reaction rate)

The curve typically shows a sigmoidal or hyperbolic shape, reaching a plateau at higher substrate concentrations.

This experiment elucidates how substrate concentration affects enzyme activity, providing insights into enzyme kinetics and saturation effects.

Time course effect on enzyme activity

Aim:

To investigate the time course effect on enzyme activity using [insert enzyme name].

Principle:

Enzyme activity is often influenced by time due to factors such as substrate depletion, product inhibition, or changes in enzyme stability over time. This experiment aims to determine how enzyme activity changes over a period of time under controlled conditions.

Materials and Methodology:

Materials:

- [List of materials including enzyme, substrate, buffers, etc.]

Methodology:

1. **Preparation of Reaction Mixtures:**
    - Prepare a series of reaction mixtures with varying incubation times (e.g., 0 min, 15 min, 30 min, 60 min).
    - Include appropriate controls (e.g., blank without enzyme) to account for non-enzymatic reactions.
2. **Incubation:**
    - Incubate the reaction mixtures at the specified temperature (optimal for the enzyme) for the designated time periods.
3. **Termination of Reactions:**
    - Stop the reactions at each time point using a suitable method (e.g., adding a stopping reagent, dilution).
4. **Measurement of Enzyme Activity:**
    - Measure the enzyme activity at each time point using a method appropriate for the enzyme (e.g., spectrophotometric assay, chromatography, pH change).
5. **Data Collection:**
    - Record the enzyme activity measurements for each time point.

Procedure:

1. **Preparation of Reaction Mixtures:**
    - Label tubes or wells accordingly for each time point.
    - Prepare the reaction mixture as per the experimental design.
2. **Incubation:**
    - Incubate the reaction mixtures in a controlled environment (e.g., water bath, incubator) at the specified temperature.
3. **Termination of Reactions:**
    - At each time point, add the stopping reagent to halt the reaction.

4. **Measurement of Enzyme Activity:**
    - Conduct the assay for enzyme activity measurement as per the protocol.
5. **Data Recording:**
    - Record the data obtained from each assay, noting the time points and corresponding enzyme activities.

Given Results (Hypothetical):

Time (min)	**Enzyme Activity (Units)**
0	100
15	90
30	80
60	60

Effect of pH on enzyme activity

Aim: To investigate the effect of pH on the activity of the enzyme [insert enzyme name].

Principle: Enzymes function optimally within specific pH ranges due to their dependence on the ionization state of amino acid residues critical for catalytic activity. Deviations from this optimal pH range can disrupt enzyme structure and function, thereby affecting its activity.

Materials and Methodology:

Materials:

- [List of materials]

Methodology:

1. **Preparation of buffers:** Prepare a series of buffers with varying pH values (e.g., pH 4, 5, 6, 7, 8, 9) using appropriate buffer solutions.
2. **Enzyme preparation:** Prepare a stock solution of the enzyme with known concentration.
3. **Experimental setup:**
    - Label a set of test tubes or cuvettes for each pH condition.
    - Add equal volumes of enzyme solution to each test tube.
4. **Incubation:** Incubate the enzyme solutions at a controlled temperature (e.g., 37°C) for a fixed period to allow the enzyme to equilibrate to each pH condition.
5. **Substrate addition:** Add a standardized amount of substrate to each tube simultaneously and start the timer.
6. **Reaction monitoring:** Measure the absorbance, color change, or any other measurable parameter at regular intervals using a spectrophotometer or other suitable method.
7. **Data collection:** Record the initial rate of reaction (e.g., initial velocity) for each pH condition.

Procedure:

1. Prepare buffers of pH 4, 5, 6, 7, 8, and 9 using appropriate buffer solutions (e.g., acetate buffer, phosphate buffer).
2. Dilute the enzyme stock solution to a suitable concentration in each buffer.
3. Incubate the enzyme solutions at 37°C for 10 minutes.
4. Add the substrate (specific to your enzyme) to each tube simultaneously and start the timer.
5. Measure the absorbance at 340 nm using a spectrophotometer every 30 seconds for 3 minutes.
6. Calculate the initial rate of reaction for each pH condition based on the change in absorbance over time.

Given Results:

pH Initial Rate of Reaction (Absorbance/min)

4 0.12
5 0.18
6 0.25
7 0.30
8 0.20

Effect of temperature on enzyme activity

Aim:

To investigate how varying temperatures affect the activity of the enzyme [Specify enzyme].

Principle:

Enzyme activity is influenced by temperature due to its effect on the enzyme's structure and the rate of enzyme-substrate complex formation. Typically, enzymes have an optimal temperature at which they function most efficiently. Above or below this temperature, the enzyme activity may decrease due to denaturation (at high temperatures) or decreased molecular movement (at low temperatures).

Materials and Methodology:

Materials:

- [List of materials such as enzyme solution, substrate solution, buffer solutions, test tubes, water bath, thermometer, etc.]

Methodology:

1. **Preparation**: Prepare enzyme solutions, substrate solutions, and buffer solutions according to standard protocols.
2. **Experimental Setup**:
    - Label several test tubes for different temperature conditions (e.g., 0°C, 20°C, 40°C, 60°C, 80°C).
    - Place each test tube in a water bath set to the desired temperature using a thermometer to monitor.
3. **Enzyme Reaction**:
    - Add a fixed volume of enzyme solution to each test tube.
    - Allow the enzyme to equilibrate to the temperature of the water bath for a few minutes.
4. **Substrate Addition**:
    - Add a fixed volume of substrate solution to each test tube simultaneously to initiate the reaction.
    - Start a timer to record the reaction time.
5. **Reaction Monitoring**:
    - At regular intervals (e.g., every 30 seconds), take out a sample from each test tube and immediately stop the reaction (e.g., by transferring the sample to an ice bath).
    - Use a spectrophotometer or other appropriate method to measure the concentration of the reaction product formed.
6. **Data Collection**:
    - Record the absorbance or other relevant data for each temperature condition at each time point.

7. **Data Analysis**:
    - Plot a graph of enzyme activity (rate of reaction or product formation) against temperature.
    - Analyze the data to determine the optimal temperature for enzyme activity and the effect of temperature on enzyme stability and activity.

Procedure:

1. Prepare all materials and set up the water baths to the desired temperatures.
2. Mix enzyme and substrate solutions in the test tubes at each temperature.
3. Record initial absorbance or other appropriate measurements.
4. Incubate for the specified time period, taking samples at regular intervals.
5. Analyze samples and record final measurements.
6. Plot data and analyze results.

Expected Result:

- Typically, the enzyme activity will increase with temperature up to a certain point (the optimal temperature).
- Above the optimal temperature, enzyme activity will decrease due to denaturation.
- Below the optimal temperature, enzyme activity will decrease due to slower molecular movement and reduced collisions between enzyme and substrate.

By following this structured approach, you can systematically investigate and analyze how temperature affects the activity of the enzyme under study.

Iso enzymes of LDH – electrophoretic separation and specific staining technique demonstration

Aim and Principle

The aim of this experiment is to separate and visualize the isoenzymes of lactate dehydrogenase (LDH) using electrophoretic separation and specific staining techniques. LDH isoenzymes are different forms of the LDH enzyme that vary in their tissue distribution and are distinguished based on their electrophoretic mobility.

Materials and Methodology

Materials:

- Polyacrylamide gel
- LDH enzyme sample (from different tissues, e.g., heart, liver, muscle)
- Electrophoresis buffer
- LDH staining solution (e.g., NADH and nitroblue tetrazolium)

Methodology:

1. **Preparation of Polyacrylamide Gel:**
    - Prepare a polyacrylamide gel suitable for electrophoresis, typically a gradient gel to achieve better separation of LDH isoenzymes based on their molecular weight and charge.
2. **Sample Preparation:**
    - Extract LDH enzymes from different tissues (heart, liver, muscle) using appropriate extraction buffers to maintain enzymatic activity.
3. **Electrophoretic Separation:**
    - Load the prepared LDH samples into wells on the polyacrylamide gel.
    - Perform electrophoresis under suitable conditions (voltage, buffer composition, duration) to separate LDH isoenzymes based on their molecular charge and size.
4. **Staining Procedure:**
    - After electrophoresis, stain the gel to visualize LDH isoenzymes.
    - Prepare a staining solution containing NADH and nitroblue tetrazolium, which reacts with LDH to form a visible precipitate.
5. **Result Interpretation:**
    - Observe the stained gel to visualize distinct bands corresponding to different LDH isoenzymes.
    - Compare the migration distances (Rf values) of the bands with known standards or literature values to identify the specific LDH isoenzymes present in each tissue sample.

Procedure and Expected Results

1. **Electrophoresis:**
   - Run the electrophoresis for an appropriate duration until the LDH isoenzymes have separated sufficiently on the gel.
   - Monitor the migration of the LDH bands visually or using appropriate imaging techniques.
2. **Staining:**
   - After electrophoresis, immerse the gel in the LDH staining solution (NADH and nitroblue tetrazolium) for a sufficient time to allow the bands to develop.
   - Rinse the gel to remove excess staining solution and observe the stained bands.
3. **Expected Results:**
   - The gel will show multiple bands corresponding to different LDH isoenzymes from various tissues.
   - For example, LDH from heart tissue (LDH-1) typically migrates differently than LDH from liver (LDH-5) due to their different compositions and structures.
4. **Interpretation:**
   - Analyze the gel to determine the relative positions (migration distances) and intensities of LDH bands.
   - Compare with known standards or literature values to identify and confirm the presence of specific LDH isoenzymes.