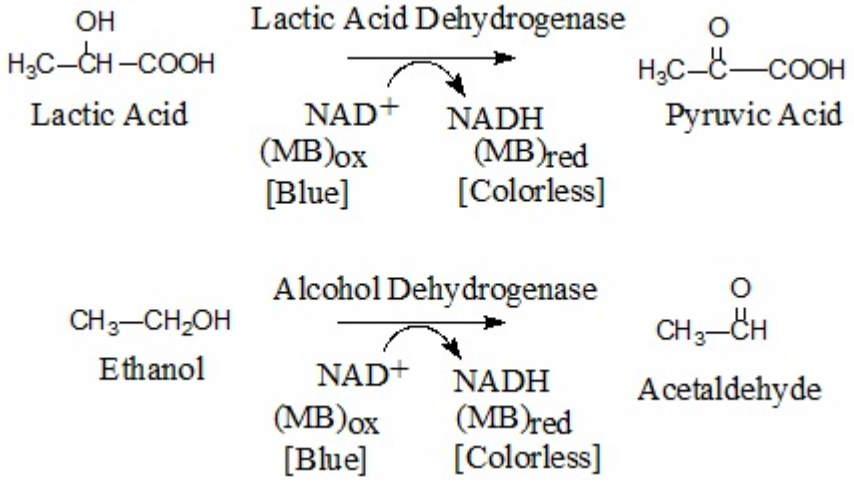
Carbohydrate Metabolism by Yeast

Experiment #12

Objective: To observe enzyme activities in respiring yeast by observing color changes in methylene blue dye as a substitute for NAD^+ in the yeast respiratory chain. You will observe the oxidation of lactic acid and ethanol by yeast lactate dehydrogenase and alcohol dehydrogenase, respectively.

Introduction

Yeast are simple organisms that can adapt to either aerobic or anaerobic conditions, and a wide range of nutrients. In this experiment you will study the activity of two enzymes, lactic acid dehydrogenase and alcohol dehydrogenase, which both use NAD^+ as the cellular oxidizing agent. You will use methylene blue as an artificial electron acceptor (oxidizing agent) in the reaction, which changes from blue to colorless as a result of its reduction in the enzyme reaction.



When methylene blue is substituted for NAD^+, the blue color of methylene blue will disappear as it is reduced and the lactic acid is oxidized to pyruvic acid, or ethanol is oxidized to acetaldehyde. NADH or reduced methylene blue can be oxidized by the mitochondrial respiratory electron transport system when oxygen is available. This will result in the blue color of methylene blue reappearing upon reoxidation by the respiratory chain. There are many poisons that can block the mitochondrial electron transport chain, notably cyanide ion (CN^-). Cyanide binds to the enzyme cytochrome oxidase, which normally reduces molecular oxygen to water in respiration. When cyanide is bound, the enzyme cannot react with oxygen, and the electron transport process (or oxidation of NADH and methylene blue) is blocked. You will demonstrate this blockage of the respiratory chain with cyanide.

Procedure

{See schematic table on next page}

1. Add 2.0 mL of yeast to a clean large (13 x 150 mm) test tube and heat it in a boiling water bath for 5 min. Allow it to cool, then place it in the warm water bath at 40°C. Label this tube as the boiled yeast tube (*e.g.*, boiled).

2. Add 2.0 mL of yeast to each of four additional clean test tubes and place them directly in the 40°C water bath (without boiling). Add 5 drops of 0.2% potassium cyanide (KCN) to **one** (only) of these test tubes. Label the tube containing KCN (*e.g.*, KCN).

3. After the solutions have had 5 min to allow the temperature to equilibrate, add 1 drop of 0.1% methylene blue solution to each of the 5 test tubes (including the one heated in boiling water in step 1). This should give each tube a distinctly blue color.

4. To the 5 test tubes add the following: add 2.0 mL of 5% sodium lactate solution to the test tube containing the boiled yeast; add 2.0 mL of 5% sodium lactate solution to the test tube containing KCN; add 2.0 mL of 5% sodium lactate solution to one test tube without KCN {label it lactate}; add 2.0 mL of 5% ethanol solution to another test tube {label it EtOH}; finally add 2.0 mL of distilled water to the last test tube {label it blank}. Be sure to label the test tubes to avoid confusing their contents. Mix each tube well, place a cork in each test tube and keep each tube in the warm water bath at about 40°C.

5. Make sure the contents are thoroughly mixed and light blue at the start, and keep the test tubes in the warm water bath at 40°C for about 10 min. Note any changes in color of the solution. Record your observations for each tube on the Report Sheet.

6. After recording your observations for the color of each solution on the Report Sheet, take note of whether there is any color at the very top of the tube. Shake each tube for a few seconds, noting whether there is any change in color as you shake the tube. If the blue color reappears, what is causing this color to reappear?

7. Remove the cork from each test tube and shake the tube. You may also try blowing air through the tube with a dropper by immersing an empty dropper in the solution and squeeze the dropper bulb. Keep squeezing the bulb as you pull the dropper out of the solution to avoid getting the solution in the dropper. If you get solution in the dropper, be sure to rinse it with water and remove all water from the dropper before blowing air into the next tube.

8. Answer all the questions on the Report Sheet.

Schematic for Test Tubes

Tube # ➜	1 (Boiled) (with Lactate)	2 (+ KCN) (with Lactate)	3 (Lactate)	4 (EtOH)	5 (H_2O blank)
Yeast Solution	Add 2.0 mL of warm yeast solution to each test tube at the start				
Boiling treatment	Heat yeast in boiling water bath for 10 min	Do Not Heat in Boiling Water			
Temperature Equilibration	Place all test tubes in a water bath at 40°C for 5 min to equilibrate				
Cyanide Solution	No KCN	5 drops KCN	No KCN	No KCN	No KCN
Methylene Blue	Add 1 drop of 0.1 % methylene blue solution to each test tube				
Substrate solution	2.0 mL of 5% Sodium Lactate	2.0 mL 5% Sodium Lactate	2.0 mL 5% Sodium Lactate	2.0 mL of 5% Ethanol	2.0 mL of deionized water
Observations	After mixing each tube thoroughly, take note of any changes in the color of each tube as a result of the reduction of Methylene Blue				

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Name _____ Section _____

Metabolism in Yeast

Experiment # 12 Pre-Lab Exercise

1. How would you classify the enzyme lactate dehydrogenase in terms of the enzyme classification scheme described in the text book, *i.e.*, oxidoreductase, transferase, hydrolase, lyase, isomerase, or ligase?

2. How would you classify the enzyme, alcohol dehydrogenase?

3. Methylene blue is a dye that is used in this experiment as an indicator for oxidation and reduction. What color is methylene blue in the oxidized state?

   What color is methylene blue in the reduced state?

4. Boiling yeast may result in these organisms no longer being able to metabolize nutrients. Explain what may be destroyed as a result of boiling.

5. Yeast are used to make beer and wine, producing alcohol in the process. In this experiment you will be adding alcohol to the yeast and a reaction will take place; the yeast will be metabolizing the alcohol, *i.e.*, consuming it. What are the conditions needed for yeast to produce alcohol and what are the conditions needed to get yeast to consume alcohol? [Hint: If fermentation takes place in air, one gets vinegar instead of wine].

6. Cyanide is a poison for humans as well as many other aerobic organisms. What is meant by aerobic?

How does cyanide act as a poison? You can find discussion of this poison in your text book regarding enzyme inhibitors.

Name _____ Section _____

Carbohydrate Metabolism in Yeast

Experiment #12 Data & Report Sheet

Lactic Acid Dehydrogenase and Alcohol Dehydrogenase Reactions in Yeast

	Observations (Color)	
	After 10 min in water bath	After shaking in air
5% Na lactate + boiled yeast		
5% Na lactate + KCN		
5% Na lactate		
Blank, distilled water		
5% Ethanol		

Questions:

1. What accounts for the differences in your observations between the blank tube containing yeast with no substrate and the tube containing 5% sodium lactate or 5% ethanol. Why does sodium lactate or ethanol result in the loss of color in this tube?

2. What accounts for the lack of any color change in the tube containing boiled yeast and sodium lactate compared to the tube containing the normal yeast and sodium lactate?

3. What is causing the blue color to reappear in the tubes after shaking them? Do all of the tubes regain the blue color? If not, explain why not.