BIO 181 Laboratory Exercise
Cellular Respiration

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Preparation

Preparation. Before completing the lab,

- read the Introduction and Box 1 in this packet;
- study sections 19.1 and 19.2 in the textbook.

1 Introduction

Life cannot exist without an external source of free energy. That energy is needed in part to make large molecules from small ones, pump molecules in solution against concentration gradients, physically move objects, fluids and gasses—in short, to drive metabolism. For plants the sun provides most of that energy. For animals the energy source exists as chemical potential energy on various organic molecules. For example, glucose is an excellent energy source. Complete oxidation of carbon in a mole of glucose under standard conditions releases 686 kcal (2870 kJ) of usable energy (that is, $\Delta G = -686$ kcal/mol). When glucose is completely oxidized, high-energy C–H bonds of organic carbon are converted to lower-energy C–O bonds of CO_2. The hydrogen ends up on water molecules, as follows:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O. \quad (1)$$

The oxygen we breathe is required to balance this reaction.

Equation (1) is a bit misleading; cells don't just "burn" glucose willy-nilly like that. If they did they would lose the energy as light and heat, and although that might be spectacular they would gain nothing. Instead, cells control oxidation of carbon very carefully to use the energy to drive the following endergonic reaction:

$$\mathrm{ADP} + \mathrm{P}_i \rightarrow \mathrm{ATP}; \quad \Delta G = 7.3\mathrm{kcal/mole}. \quad (2)$$

In essence, then, cells use free energy released from glucose oxidation to harvest chemical potential energy in the form of phosphate bonds on ATP.

The mechanism by which cells use equation (1) to power equation (2) consists of a sequence of biochemical pathways called, essentially in order, **glycolysis** (literally, "loosening sugar"), **pyruvate oxidation**, the **Krebs cycle** (also called the citric acid cycle or tricarboxylic acid cycle) and finally the **electron transport chain** and **oxidative phosphorylation**. In this exercise, we will investigate the properties of one enzyme, **enolase**, catalyzing one step in a portion of glycolysis. We use as our study organism baker's and brewer's yeast (*Saccharomyces cerevisiae*). Box 1 outlines glycolysis and subsequent fermentation in this organism.

Box 1: Glycolysis and fermentation in yeast

Yeast is a single-celled fungus that bakers and brewers use to make people fat and drunk, respectively. Like all organisms, energy metabolism in yeast is driven by glycolysis, which converts glucose to pyruvate as follows:

$$\text{Glucose} \rightarrow (\text{5 steps}) \rightarrow 2\ \text{G3P} \rightarrow (\text{3 steps}) \rightarrow 2\ \text{2PG} \rightarrow 2\ \text{PEP} \rightarrow 2\ \text{PYR},$$

where G3P is glyceraldehyde 3-phosphate, 2PG is 2-phosphoglycerate, PEP is phosphoenolpyruvate, and Pyr is pyruvate. (See Fig. 1 for details.) The fate of pyruvate, at least in yeast, is determined by availability of oxygen. If oxygen is available, then yeast complete the oxidation of pyruvate, converting it to $6CO_2$ and $6H_2O$. In the absence of O_2, however, yeast send pyruvate to the fermentation pathway, which does the following:

$$2\ \text{PYR} + 2\text{NADH} \rightarrow 2\ \text{ETOH} + 2\ CO_2 + 2\text{NAD}^+,$$

where ETOH is ethanol and NAD^+ is nicotinamide adenine dinucleotide. To make beer, brewers feed yeast sugar (maltose) in an oxygen-poor environment. The CO_2 given off by fermentation makes beer foamy and the ETOH makes people drive erratically.

Today we will study step 9 of glycolysis (Fig. 2), which is catalyzed by enolase. If enolase functions properly, then pyruvate will be formed. If there is no oxygen, then the pyruvate will be converted to CO_2 and ETOH. We will use the former as an indicator of the rate at which pyruvate is formed, and therefore the activity of enolase.

2 Experimental Details

ln the past, esearchers working with enolase discovered that it's a fairly tricky enzyme to work with. Sometimes it works, sometimes it doesn't. One research group, led by Drs. Barnes and Nobel, suggested that when it doesn't work, a unknown cofactor is missing from the experimental solution. That is, they suggest that enolase requires a change of shape to expose its catalytic site. This requirement is common and often is produced when a **cofactor** binds to the enzyme. The cofactor is frequently an ion. Barnes and Nobel proposed this hypothesis:

Hypothesis: Barnes and Nobel

Barnes and Nobel suspect that enolase requires magnesium ions (Mg^{2+}) as a cofactor.

In previous semesters, students tested this hypothesis in the lab. They grew yeast cultures in fermenting tubes designed to capture CO_2 released as the cells go through fermentation. **The amount of CO_2 captured in the fermenting tube is proportional to the activity of enolase, all else being equal** (see Box 1). They varied Mg^{2+} concentration by diluting a stock solution of $MgSO_4$ and, in some cases, adding NaF, which is known to cause Mg^{2+} to precipitate out of solution in the form of insoluble magnesium fluoride. This therefore removes Mg^2+ from solution and makes in unavailable as a cofactor.

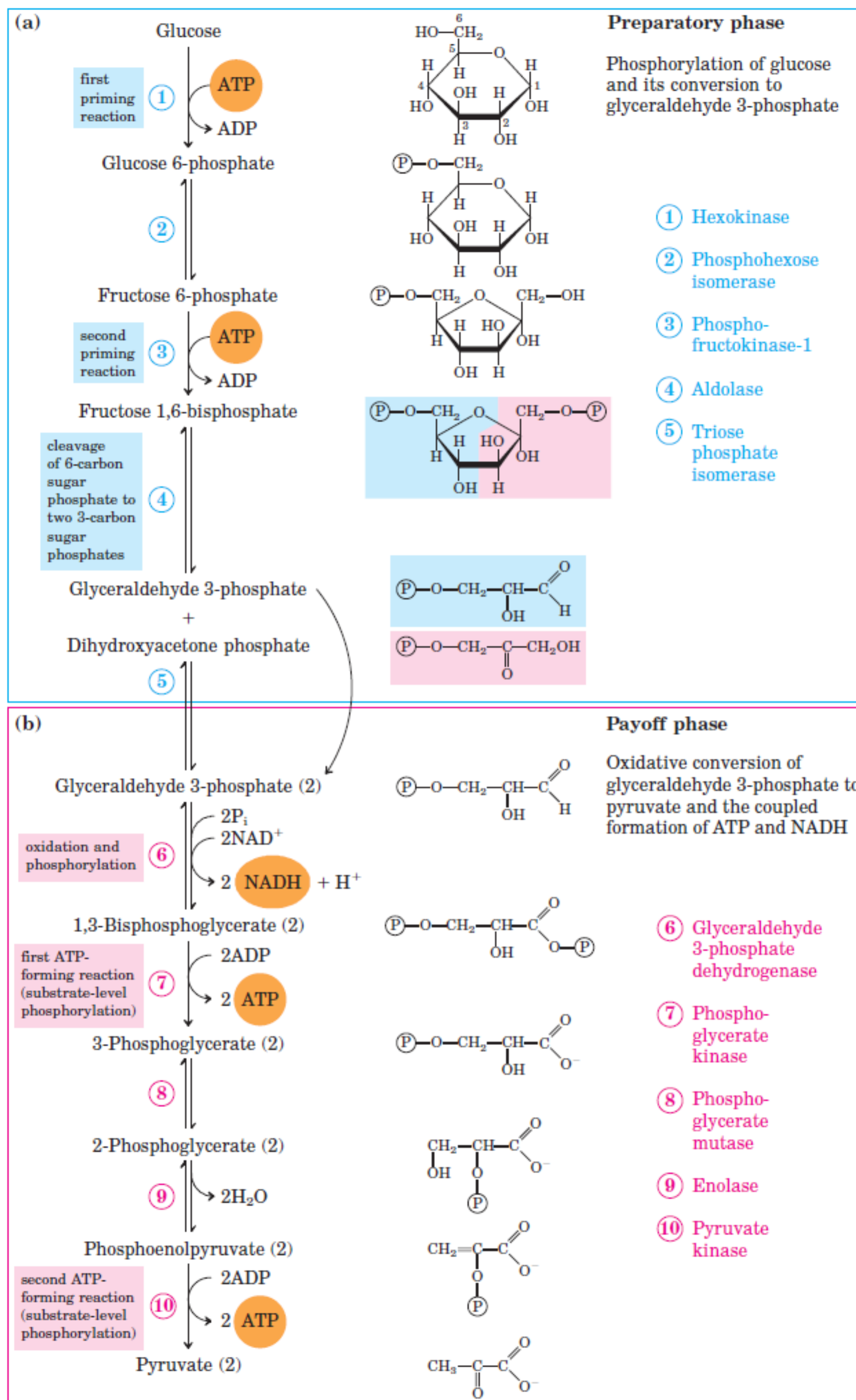

Figure 1: The Embden-Meyerhof-Parnas (glycolysis) pathway. Today we study step 9 (Fig. 2).

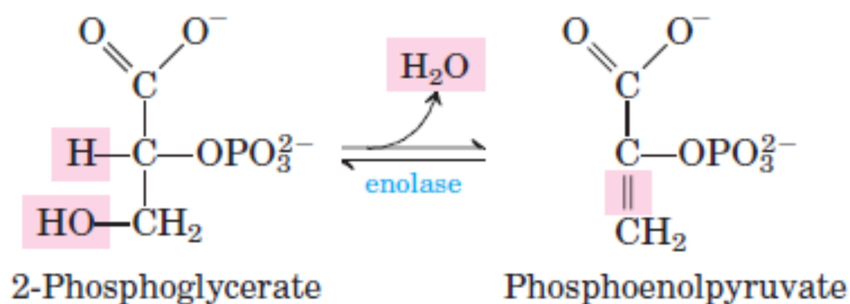

Figure 2: The action of enolase in step 9 of glycolysis.

Table 1: Solution recipes for preparation of ***test tubes*** for enolase experiment.

Tube #	Na-PYR	0.2M NaF	0.2M $MgSO_4$	H_2O
1	—	—	5 ml	5 ml
2	—	—	3 ml	7 ml
3	—	—	1 ml	9 ml
4	—	5 ml	5 ml	—
5	—	5 ml	3 ml	2 ml
6	—	5 ml	1 ml	4 ml
7	5 ml	2.5 ml	—	2.5 ml

Summary of Student Procedures

Starting with 7 clean testtubes and fermenting tubes, the students performed the following procedures.

1. They prepared 7 test tubes as described in Table 1 .
2. They added 20 ml of yeast suspension (standard baker's yeast in 5% glucose solution) to each of 7 **fermenting tubes** as shown in Table 2.
3. The contents of each test tube prepared in step 2 was added to the yeast suspension in the appropriate fermenting tube, as specified in Table 2.
4. Fermenting tubes were then incubated in a 37°C water bath for 15 minutes.
5. Gas in the capture arm of the fermentation tubes (the closed tube on the left side of the picture in Table 2) was evacuated, and tubes were returned to the 37° bath. Students then began measuring the amount of CO_2 collected in the capture arm every 5 minutes for 40 minutes.

Table 2: Preparation of ***fermenting tubes*** for the enolase experiment.

Tube #	Yeast	Tube 1	Tube 2	Tube 3	Tube 4	Tube 5	Tube 6	Tube 7
1	20 ml	10 ml	—	—	—	—	—	—
2	20 ml	—	10 ml	—	—	—	—	—
3	20 ml	—	—	10 ml	—	—	—	—
4	20 ml	—	—	—	10 ml	—	—	—
5	20 ml	—	—	—	—	10 ml	—	—
6	20 ml	—	—	—	—	—	10 ml	—
7	20 ml	—	—	—	—	—	—	10 ml

3 Experimental results

The experimental data are available from the course website as an Excel spreadsheet. You can import them into any spreadsheet software, including but not limited to Excel (any platform), Numbers (on Mac), Google Sheets (online, cloud-based) and Open Office Calc (free, open source). It does not matter which one you use, but you will need access to a spreadsheet software package.

The data set is organized in two sheets:

1. **Sheet 1—Raw Data:** Contains volumes of CO_2 captured for each tube in every 10 groups of students. Data were obtained in 5 minute intervals for 40 minutes. Numbers are in cubic mm. (Note: $1\,\mathrm{mm}^3 = 1\,\mu\mathrm{l}$.)
2. **Sheet 2—Summary Stats:** Contains summary statistics for the raw data, organized into 3 tables.
   (a) *Upper table:* Averages for all 10 groups at each time point.
   (b) *Middle table:* Standard deviations for all 10 groups at each time point.
   (c) *Lower table:* Standard errors of the mean for all 10 groups at each time point. The formula for standard error is:
   $$\frac{s}{\sqrt{n}},$$
   where s is standard deviation and $n = 10$ is the sample size.

4 Analysis

Carefully study the hypothesis and experimental design. Analyzing and interpreting the data will require you to connect the experimental procedures to the hypothesis being tested; that is, you will have to work out why the tubes were set up as they were.

Complete your analysis by answering the questions on Canvas.

Example 1: Calculating concentrations of dilutions

Recall from basic chemistry that one can easily find the concentration of a dilution using a formula of the form,

$$C_1 V_1 = C_2 V_2,$$

where C_1 and V_1 are initial concentration and volume, respectively, and C_2 and V_2 are final concentration and volume.

Problem: Calculate the final concentration of Mg^{2+} after 3 ml of a 0.6 M solution of $MgSO_4$ is diluted with 6 ml of water.

SOLUTION: We know we're after C_2, so we solve the equation above to get

$$C_2 = \frac{C_1 V_1}{V_2},$$

where $C_1 = 0.6$ M, $V_1 = 3$ ml, and $V_2 = 9$ ml. (Note: we have to add the original volume to the volume added to obtain final volume.) Therefore,

$$C_2 = \frac{0.6\,\mathrm{M}\,(3\,\mathrm{ml})}{9\,\mathrm{ml}} = \frac{0.6}{3}\,\mathrm{M} = 0.2\,\mathrm{M}.$$

Note 2 things:

1. We are required to perform the proper dimensional analysis. Note how the ml units cancel and we're left only with a unit of concentration, molar (= M), as we should.
2. Notice that how I laid the calculation out makes the solution easily calculated without a calculator. Patterns like that are always things to look for.