BIOL/CHEM 3361 Biochemistry I
(and BIOL 6352)
Spring 2016

Due: Mon., Apr. 4th at 5:00 pm in the collection box in the hall outside FO 3.606
(*No late Problem Sets will be accepted.)*
(To void last minute difficulties, you may turn them in early -- at lecture or dropped in the collection box.)

PROBLEM SET 3

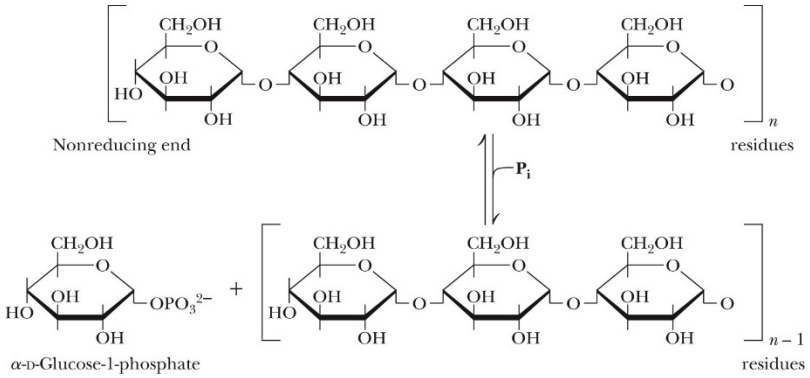
There are 10 problems in the problem set 3. Each problem will be graded on a 10 point scale, for a total of maximum 100 points.

For full credit, **all steps** to the solutions of the following problems must be shown. You may work together on the problems, but you may not copy or plagiarize. Your answers must be written, must show your solution steps and/or formulas when requested, and must be in your own words.

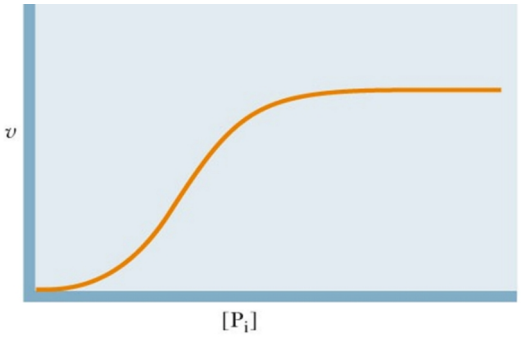
Problem 1

Glycogen phosphorylase converts glycogen into glucose subunits and is the rate limiting step in glycogenolysis (that will be treated later in the course).
It catalyzes the following reaction:

$(\alpha\text{-}1,4 \text{ glycogen chain})_n + Pi \rightleftharpoons (\alpha\text{-}1,4 \text{ glycogen chain})_{n-1} + \alpha\text{-D-glucose-1-phosphate}$



The plot below indicates the rate of the reaction for glycogen phosphorylase as function of phosphate concentration:



a) Explain whether glycogen phosphorylase follows classic Michaelis-Menten kinetics or not? Explain in detail your conclusion.
b) Based on the plot, do you expect the enzyme to be a monomer or a dimer (note that a single glycogen phosphorylase chain has a single Pi substrate binding site)?
c) Caffeine is an allosteric inhibitor and glycogen phosphorylase is a K-system. How is the rate curve affected and why?
d) Plot the rate versus substrate curve for the enzyme in the presence of 1) ATP 2) Glucose 3) AMP, knowing that the first 2 are feedback inhibitors while AMP is a positive effector.
e) Draw the plot of the v versus substrate concentration for potential effectors in V-systems, and explain the rationale behind their effect on Vmax and K_M (or $K_{0.5}$).

Problem 2

Repeated vomiting and severe dehydration cause alkalosis, a condition in which serum pH is higher than normal (7.45 or higher). Contrarily acidosis, occurring for example in patients with renal failure, occurs when the serum pH fall below 7.35.

a) Plot the curves of hemoglobin oxygen saturation for patients in conditions of alkalosis or acidosis, and compare it to the one in normal conditions.
b) Explain why alkalosis can result in hypoxia even with sufficient O_2 in the blood?
c) In acidosis is oxygen released more or less efficiently to the tissues? What is the effect of acidosis on hemoglobin in the lungs?
d) Fetal hemoglobin possess a higher affinity for O_2 by possessing two α and two γ subunits instead of two α and two β subunits. Explain why fetal hemoglobin shows an exacerbated Bohr Effect compared to adult hemoglobin.

Problem 3

1) Draw and name the following monosaccharides

a) Anomer of β-D-galactose
b) Enantiomer of D-galactose
c) The pyranose and furanose forms of D-galactose
d) The epimer of D-galactose
e) One of β-D-galactose diasteroisomer

f) the corresponding aldopentose of D-talose with the same stereochemistry at each chiral (C-1) center

2) Determine the relationship between the following pairs of sugars (anomers, enantioners, epimers, diasteroisomers, aldose and ketose pair, others (define the relationship):

a) D-Ribose and L-Ribose

b) D-Arabinose and D-ribulose

c) D-mannose and D-talose

d) 2-deoxy-α-D-ribose and D-ribose

e) Glucose and maltose

f) α-D-galactopyranose and α-D-galactofuranose

g) β-D-galactopyranose and α-D-galactopyranose

h) D-psicose and D-tagatose

Problem 4

1) Allolactose is a disaccharide similar to lactose that differ only in the position of the glyosidic bond (1,6 instead of 1,4).

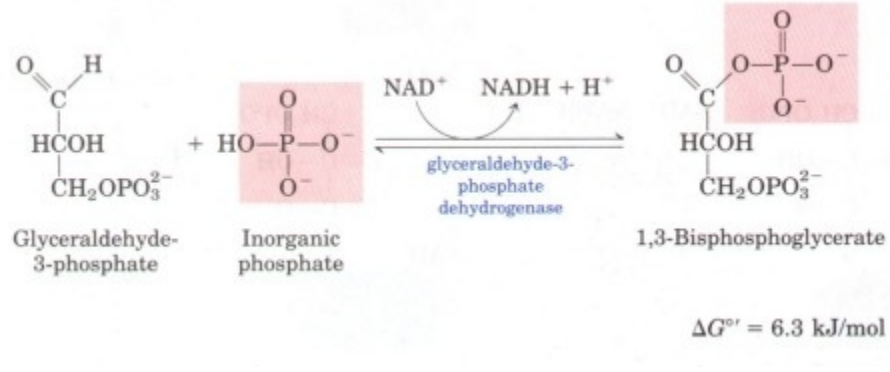
a) Explain whether allactose is a homo or heterodisaccharide?
b) Draw the structure of lactose and allolactose and highlight the glycosidic bond. In both structures highlight the anomeric carbon (write it as mixture of the 2 anomers) and the non-reducing end.
c) Allolactose can be a substrate of β-galactosidase as lactose. Draw the products of the enzymatic reactions (assuming mutarotation in aqueous solution).

2) Raffinose is a trisaccharide composed of α-D-galactose, D-glucose and D-fructose. It can be found in several vegetables including beans, cabbage, broccoli, asparagus, other vegetables, and whole grains. Raffinose can be hydrolyzed to D-galactose and sucrose by the enzyme α-galactosidase, an enzyme not found in the human digestive tract.

a) Draw the structure of raffinose and the product of the reactions of alpha-galactosidase (the hydrolyzed glycosidic bond is 1,6 - assume mutarotation in aqueous solution)
b) After digesting of raffinose with alpha galactosidase the products of the reaction are treated with sucrase. Draw the final products of the reaction in the mixture in acqueous solution.
c) Indicate which of the products are aldoses or ketoses.

Problem 5

Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) is a tetrameric enzyme that catalyzes one of the steps of glycolysis.



A new colorimetric GAPDH assay provides a non-radioactive method to measure the enzyme activity of GAPDH, based on the oxidation an alternative substrate, D-glyceraldehyde (note that with D-glyceraldehyde the enzyme activity is lower than the substrate D-glyceraldehyde3-phosphate).

NADH (MW 633.4 g/mol) absorbs light at 340 nm while NAD^+ does not show any absorption at that wavelength.

The Beer-Lambert Law relates the absorbance of a solute to its concentration:

$A = \varepsilon.c.L$

A is absorbance, ε is the molar extinction coefficient, c is concentration, and L is the path length of the solution in the spectrophotometer (usually in cm).

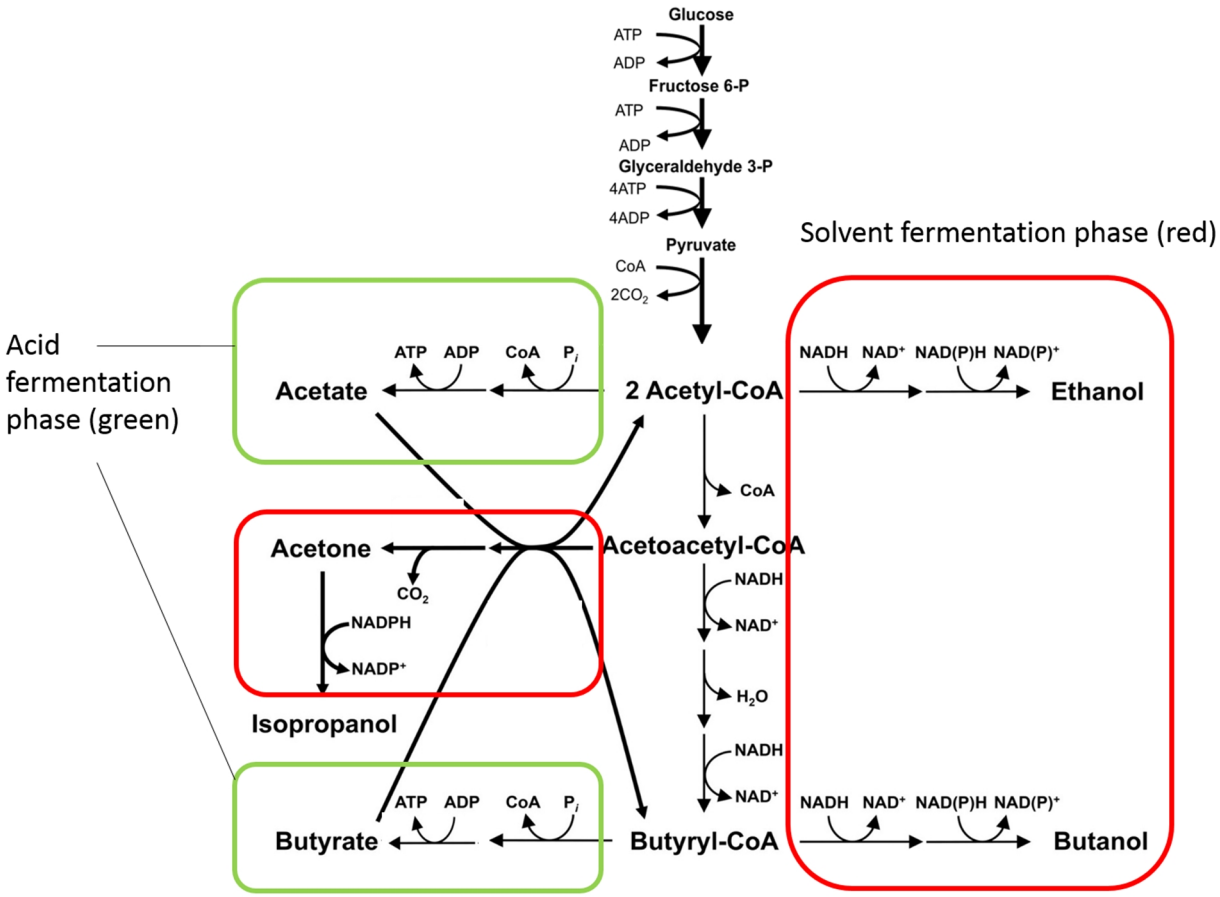
In the lab you have available NADH. You dissolve 9.951 mg in 100 ml of buffer and measure an absorbance of 0.4725 at 340 nm in a 0.5 cm cuvette.

Subsequently in a 1 cm cuvette, you mix 25 µl of 20 mM NAD^+, 25 µl of D-glyceraldehyde 0.2 M , 400 µl of TAE (pH=8.6), and 50 µl of a solution containing GAPDH at a concentration of 12 mg/l. You place the cuvette in a spectrophotometer and follow the kinetics of absorbance at 340 nm. You measure a rate of decrease of absorbance (ΔA) of 0.43 min^{-1}.

a) Determine the absolute amount of NAD^+ consumed after 30 s in the reaction (in nmol)
b) Determine the specific activity of GAPDH in NADH produced per GADPH (in µmol/mg/min)

Problem 6

Certain *Clostridium* strains can anaerobically ferment pyruvate to ethanol, butanol and acetate (ABE fermentation) following this scheme:

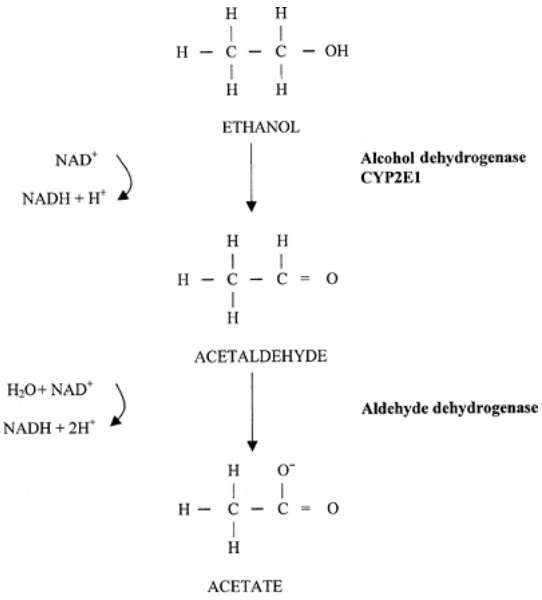


a) The fermentation pathway marked in red is called solvent fermentation phase. In this solvent fermentation phase butanol, ethanol and acetone are generated. Explain the role of the solvent fermentation phase as metabolic pathway in respect to glycolysis.

b) What is the main difference in terms of metabolic energetics between solvent fermentation phase and the acid fermentation phase?

Problem 7

Ethanol is metabolized by alcohol dehydrogenase and aldehyde dehydrogenase to acetate, which can be susequently converted to acetyl-CoA.



Ethylene glycol (an automotive antifreeze) is poisonous and oxidized to oxalic acid (as oxalate), a toxic compound found in rhubarb leaves and many other plants. Ethylene glycol is metabolized by the same enzymes as ethanol, producing glycolate which is further oxidized to oxalate and not further metabolized.

a) Write the reactions involved in Ethylene glycol conversion and explain also why its metabolism is not energetically favorable.
b) Can ethylene glycol poisoning be prevented by intoxicating concentrations of ethanol? If yes, why?

Problem 8

Deficiency of pyruvate kinase in the red blood cells can result in insufficient absorption of the right amount of O_2. Explain which intermediate of the glycolysis is responsible for the observed effect and why? Draw the substrate and product of the enzymatic reaction generating that intermediate.

Problem 9

A mutation in Phosphofructokinase causes the loss of the allosteric site for ATP. Which effect on glycolysis is expected? Explain in detail.

Explain which of the following mutations would result in the opposite effect on the rate of glycolysis in liver:

a) a mutation causing the loss of the binding site for citrate in phosphofructokinase

b) a mutation causing the loss of the binding site for fructose 1,6-bisphosphate in pyruvate kinase.

c) A mutation decreasing the activity of adenylate kinase

Problem 10

In the laboratory you are performing an experiment by growing yeast (*S. cerevisiae*) in strictly anaerobic conditions, using ^{14}C labeled glucose as carbon source. You isolate the products of the fermentation after you give enough time for each intermediate in the pathway to become labeled. You are able to isolate ethanol exclusively labeled on C-1.

a) Determine position of the labeled carbons on the glucose used for the fermentation.

b) In a second experiment, you use ^{14}C labeled glucose at position 3. Which labeled products you expect to isolate upon fermentation?