

1. A Draw the structure of glucose using either a ring or straight chain representation. Explain how (i) mannose and (ii) galactose differ from glucose. Which are (a) epimers; (b) enantiomers; (c) diastereomers?

Take glucose from the notes. Reverse orientation at C2 for mannose and at C4 for galactose. Glucose and mannose & glucose and galactose are epimers-but not mannose and galactose. None are enantiomers (mirror images) all are diastereomers (differ in one chiral center not C5).

B. A hypothetical hexasaccharide was subjected to chemical analysis and the following data obtained:

- a.) No reaction was obtained with Fehling's reagent.
- b.) Following hydrolysis with dilute acid only glucose could be detected.
- c.) Upon methylation and hydrolysis the reaction mixture contained

2	2,3,4,6 tetramethyl glucose
4	2,3, 6 trimethyl glucose.

What can you deduce about the structure of the hexasaccharide?

- a) no reducing sugars so no free CHO (or C1 not in glycosidic link)
- b) hexaglucose
- c) G(1-4)G(1-4)G(1-4)G(1-4)G(1-1)G i.e. a normal chain terminated by the last glucose using C1 and not C4

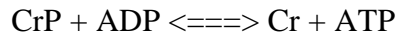
C What do you expect would be the overall shape of a polysaccharide that has alternating (1 4) and (1 4) glycosidic bonds? Explain your answer.

(1 4) have a 60 kink and if only this bond is present we would get a spiral. (1 4) glycosidic links are "linear" So a simple answer would be a spiral in which the diameter is two G resides and not 1. However a more thorough answer would point out that in the (1 4) glycosidics the second glucose is flipped w.r.t. the first so the 60 bend of the next alpha link is in the opposite direction to the predecessor. The result would be a "saw-tooth".

2. A. The free energies of hydrolysis of glucose-6-phosphate and fructose-6-phosphate are both -13.8 kcal/mole. What is the equilibrium constant for the reaction catalyzed by phosphohexose isomerase (G-6-P \rightleftharpoons F-6-P)?

		Go'
	G-6-P \rightarrow G + P	-13.8 kcal/mole
	F + P \leftarrow F-6-P	+ 13.8
Net:	G-6-P + F \rightleftharpoons G + F-6-P	0
Using:	G \rightleftharpoons F	-218.8 - (-219.2) = 0.4
Final:	G-6-P \rightleftharpoons F-6-P	0.4
	Use $Go' = -RT \ln K$ with $Go' = 0.4$ $K = 0.5$	

B. Calculate G at 37°C for the reaction



under conditions that actually exists in the cytoplasm of neurons, i.e. CrP = 4.7 mM, Cr = 1.0 mM, ADP = 0.2 mM and ATP = 2.6 mM (Cr = creatine, CrP = creatine phosphate).

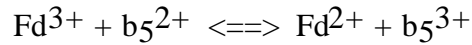
$$Go' = -10.4 + (-7.4) = -3 \text{ kcal/mole}$$

$$G = -3.0 + RT \ln \frac{[1 \times 10^{-3}] [2.6 \times 10^{-3}]}{[4.7 \times 10^{-3}] [2 \times 10^{-4}]}$$

using 0.6 for RT to get answer is in kcal/mole. Note that because the number of terms in numerator and denominator are the same any conc. unit will work as long as it is used for all species.

C. Ferredoxin (Fd) is an iron containing protein present in the photosynthetic apparatus; during photosynthesis the iron undergoes a 1-electron redox reaction with an $E^{\circ} = -0.42\text{v}$. Similarly cytochrome b5 is an iron-protein present in the microsomes with an $E^{\circ} = 0.0\text{ v}$.

For the reaction at pH = 7



Where does the equilibrium lie? Why?

- (a) Very much to the right?
- (b) A little to the right?
- (c) About in the middle?
- (d) A little to the left?
- (e) Very much to the left?

Fd has much the more negative redox potential so relative to b5 it is a very good e-donor and a poor e-acceptor. So the reaction will lie very much to the left (e).

D. It is possible to prepare cell sacs in which the internal contents have been replaced by a solution of your choice. You fill the sac with 400 mM KCl and 10 mM NaCl. You place this sac into a medium with the composition 10 mM KCl + 400 mM NaCl, What is the membrane potential that is immediately developed at 37°C?

Using the Goldman equation the numerator in the argument to the log term is

$$1 \times [0.01] + 0.01 \times [0.4] + 0.5 \times [0.41]$$

and the denominator is

$$1 \times [0.4] + 0.01 \times [0.01] + 0.5 \times [0.41]$$

Divide, take the natural log and multiply by RT/F where $T = 273+37$.

3. Because grapes make their own sugar it occurred to Gallo Vineyards that the fermentation of grape juice to wine by glycolysis could proceed effectively without the participation of sugar phosphates. This being the case they hired Creative Biochemists Inc. to design an improved glycolytic pathway for the formation of alcohol from glucose. CBI investigated this problem and reported that such a pathway could be designed: this pathway is characterized by being significantly shorter but would require that the **specificity** (but not the **reaction catalyzed**) of all remaining enzymes would have to be changed.

Using chemical structures or names propose such a pathway. Name the enzymes that you utilize

Does the resulting **overall** reaction differ in any way from traditional glycolysis?

Glucose --*hexose isomerase*-->fructose

fructose ---*aldolase*---> dihydroxy-acetone + glyceraldehyde.

dihydroxy-acetone --*triose isomerase*---> glyceraldehyde

glyceraldehyde ---*glyceraldehyde dehydrogenase*--> 1-phosphoglyceric acid

1-phosphoglyceric acid---*phosphoglycerate kinase*---> glyceric acid

glyceric acid ---*enolase*---> enol-pyruvate

enol-pyruvate ---non-enzymic---> pyruvate.

We have eliminated 4 enzymes: hexokinase, phosphofrucokinase, phosphoglycerate mutase and pyruvate kinase. The remaining 6 have their specificities changed to reflect the missing P groups.

glucose + 2 ADP + 2 P_i + 2 NAD <====> 2 pyruvate + 2 ATP + 2 NADH

This is the same as conventional glycolysis.

4. You set up the following incubations with fresh yeast juice which has been thoroughly dialyzed. In each case deduce the compounds which accumulate in the reaction mixture.

a) Glucose with an equal molar amount of ATP.

F-6-P or better a mixture of G-6-P and F-6-P

b) Glucose-1-phosphate with an equal molar amount of ATP.

DHAP + 3PGA or better F-1,6 bisP because eqm of aldolase is towards condensation not cleavage.

c) Dihydroxyacetone phosphate + NADH.

A mixture of DHAP + 3PGA; latter needs NAD to be further metabolized.

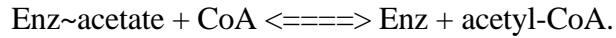
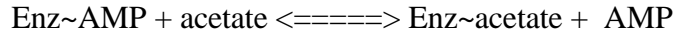
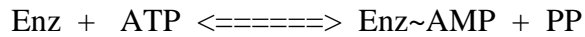
d) Fructose bisphosphate + NAD^+

A mixture of DHAP + 3PGA; latter needs P (+NAD) to be further metabolized.

e) Glucose with excess ATP and an equal molar amount of NAD^+

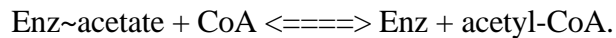
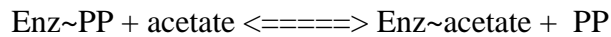
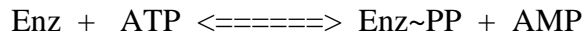
Essentially same as (d)

5. Your neighborhood enzymologist has just purified a new enzyme which synthesizes acetyl CoA from acetate + ATP. At equilibrium the reaction mixture contains some AMP + PP. The following 3-step mechanism is proposed:



Using exchange reactions how would you:

(1) Distinguish this reaction from the plausible alternative



(2) Verify the individual steps of the first sequence.

(1) Demonstrate that in the absence of acetate and CoA the exchange $\text{PP} \rightarrow \text{ATP}$ exists but not $\text{AMP} \rightarrow \text{ATP}$.

(2) Demonstrate the following exchanges

Exchange	Extra additions necessary to demonstrate the exchange
$\text{PP} \rightarrow \text{ATP}$	None
$\text{AMP} \rightarrow \text{ATP}$	acetate
$\text{CoA} \rightarrow \text{Acetyl CoA}$	None
$\text{acetate} \rightarrow \text{Acetyl CoA}$	AMP

Note that 2 and 4 are redundant but will be given in a complete answer.