Biochemistry I, Spring Term 2000 - Third Exam:Solution Key:

Section A: Multiple choice:

1-c

2-b (1/2 points given for a and c)

3-a (1/2 point given for d)

4-b

5-c

6-c (1/2 point given from d)

7-b (1 point given for c, 1/2 point given for d)

8-b

9-c (1/2 point given for b or d)

10-c

11-b or d

12-b

13-b

14-b

Section B:

B1: Here is my solution (not necessarily unique):

Step #	Separation Technique	Basis of Separation	Protein(s) retained for next step	Protein(s) discarded by this step
1	gel filtration	molecular weight	hexose kinase G-3-P dehydrogenase	glycogen phosphorylase Phsophofructose kinase
2	NAD affinity chromatography	Binding of G-3-P dehydrogenase to the NAD on the column	hexose kinase	G-3-P dehyd.

Note; to get full marks you had to specify the **type** affinity column, ie glucose, G-3-P, ATP, NAD etc.

B2.

i) Can Glucose-1-P be used to efficiently phosphoryate ADP to make ATP under standard conditions

The two half-reactions are:

G-1-P ---> G+P$_i$ ΔG^0 = -20.9 kJ/mol

ADP+P$_i$ ---> ATP ΔG^0 = +30.5 kJ/mol

Which give as a sum:

G-1-P + ADP ---> G + ATP ΔG^0 = +9.6 kJ/mol

Since the ΔG^0 is positive, the equilibrium position of the reaction will lie far to the left, i.e. favouring high concentrations of G-1-P and ADP and low concentrations of ATP.

ii) *Assuming that the intracellular concentration of ADP and ATP are 1mM, calculate the ratio of [glucose] to [glucose-1-P] such that the reaction described in part i becomes spontaneous.(4 pts)

$\Delta G = \Delta G^0$+RT ln[Glucose][ATP]/[G-1-P][ADP]

If you calculate the concentrations such that ΔG=0, then a slight increase in the concentration of G-1-P will make the reaction spontaneous.

0 = +9.6 +(2.5)ln[G](1mM)/[G-1-P](1 mM)

-3.84 = ln[G]/[G-1-P]   Therefore: [G]/[G-1-P]=0.02, or [G-1-P]/[G]=46. If the ratio of [G-1-P]/[G] exceeds 46 the reaction will become spontaneous.

B3 (6 pts): *The membrane potential across the mitochondrial membrane is approximately -150 mV (inside negative). The oxidation of each NADH pumps about 8 protons across this membrane. This generates a pH difference of 1 pH unit and stores about 160 kJ/mol of free energy (i.e. 20 kJ/mol per proton). Now, assume that the energy from NADH oxidation was used to pump phosphate ions across the membrane instead, giving an identical concentration gradient. Calculate the free energy stored by the phosphate gradient. Assume that the membrane potential is unchanged (i.e. remains at -150 mV) and the phosphate is entirely in the *doubly* ionized form (i.e. $HPO_4^=$).

The total free energy associated with this gradient is:

$\Delta G = RTln[X_{IN}]/[X_{OUT}] + ZF\Delta\psi$

$\Delta G = (2.5)ln(10)/91)+(-2)(96.6)(-0.15)$

$\Delta G = -5.75+28.3$

$\Delta G = +23.05$

Note: The concentration difference gives the same amount of free energy difference for protons and phosphate ions. In both cases it would be favorable for the proton or the phosphate to diffuse back into the cell. In the case of the electrostatic contribution, the effect is just the opposite. Not surprising since the charge on the phosphate is negative, while that on the proton is positive. Overall, the pumping of phosphate to the **outside** is favorable.

B4 (4 pts): The catalytic mechanism of lysozyme requires that Asp53 is ionized and that Glu35 is not. A mutant lysozyme was made such that Asp53 was replaced with Asn. Would this enzyme still be active? Why? Do you expect the activity to be the same, decrease, or increase? Justify your answer.

Asp53 stabilizes the carbocation. It is not essential for activity, but replacement of it with Asn would reduce the activity.

B5 (4 pts) Answer **one** of the following two questions:

i) What are the similarities and differences between product inhibition and feedback inhibition.
Both are involved with the control of metabolic pathways. The first describes inhibition directly by the product, the other describes inhibition by a metabolite, intermediate, or final product further down the pathway.

ii) What are the similarities and differences between substrate level phosphorylation and oxidative phosphorylation.
Both involve the generation of ATP. In the first case the 'high' energy potential of on compound is directly transferred to ADP to generate ATP. In the other, the energy is stored in a proton gradient, which is then used to generate ATP.

B6 (10 pts): Choose **one** of the following compounds: NADH, NAD^+, ATP, or AMP.
ii) Do high levels of this compound indicate high or low energy reserves in the cell. Why?(3 pts)

NAD+ and ADP indicate low energy levels since the energy of ATP hydrolysis is used to drive many biological processes. Low levels of NAD+ indicate that additional ATP cannot be generated by oxidative phosporylation.

NADH and ATP indicate high levcls. The NADH can be used to generate ATP.

iii) Briefly discuss how the compound you chose regulates the complete oxidation of glucose (i.e. the pathways of glycolysis, TCA cycle, electron transport, oxidative phosphorylation). Where possible give at least one specific example of an enzyme and describe how it is regulated by the compound of your choice.(7 pts).

Refer to the study guide for the last exam, as well as the lecture notes. In general high levels of ATP and NADH inhibit.

A brief summary is as follows:

Pathway	NADH/NAD+	ATP/ADP
Glycolysis	no role	PFK is the most imporant
Pyr-->Acetyl CoA	Pyruvate Dehydrogenase(inhibited by NADH)	Pyruvate Dehydrogenase(inhibited byATP)
TCA	Citrate Synthase, α-ketoglutarate DH, isocitrate DH	Citrate Synthase, α-ketoglutarate DH, isocitrate DH
Electron Transport	not regulated directly	not regulated directly

B7 (2 pts) Three biochemical reactions are shown below. Circle the one that is **NOT** an example of a redox reaction.

 The middle one is not an example of a redox reaction (the double bond has the same redox state as the alcohol)
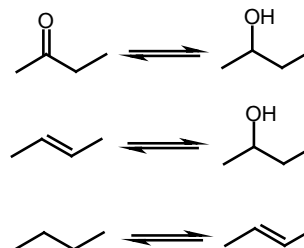
B8 (6 pts): The following is a list of enzymes that catalyze spontaneous, or energetically downhill, reactions that we have encountered in glycogen metabolism, glycolysis, regulation of glycolysis, or fatty acid metabolism:

a) Glycogen phosporylase
b) Fructose 1,6 bisphosphatase
c) Fructose 2,6 bisphosphatase
d) Thiolysis (last step in fatty acid oxidation, producing Acetyl-CoA)

For **one** of the above, answer the following:
ii & iii) What is the reverse reaction and energy used:
  a) Glycogen Synthase, using UDP-glucose
  b) Phosphofructose kinase, using ATP
  c) Phosphofructose kinase-2 using ATP
  d) Condensation of malonyl-CoA with growing fatty acid. CO_2 released, or ATP used to generate malonyl CoA.

iv) Briefly discuss how the forward and reverse reactions are coordinately regulated. If they are not regulated, say so!
  a) phosphorylation increases phorporylase activity and decreases synthase activity.
  b) F2,6P activates PFK, inhibits FBPase
  c) Phosphorylation activates FBPase2 and inhibits PFK2 (liver tissues)
  d) None.

B9: (16 pts) The following diagram is an overview of glucose oxidation under **aerobic** conditions. Note that this diagram is incomplete; for example, CO_2 generation from the TCA (Krebs) cycle is not shown.

i) Fill in the **six(6)** empty squares with the name of the appropriate molecule. (9 pts).

1: Pyruvate
2: Acetyl CoA
3: NADH

4: $FADH_2$ or succinate
5: Oxygen
6: Water

ii) Indicate, by circling and labeling the appropriate sections of the above diagram, which segment corresponds to glycolysis and electron transport. Please circle the segments in a neat fashion so that there is no ambiguity when grading! (2 pts).

Glycolysis would involve steps from glucose to pyruvate, inclusively.

Electron transport are the steps involving boxes 3,4,5, and 6 as well as the mitochondrial membrane.

iii) Briefly describe the molecular mechanism by which the proton gradient is coupled to ATP generation (ie. describe how the F_1F_oATPase converts the proton gradient to chemical energy) (3 pts)

The key points are this:
i) The enzyme has three β-subunits whose conformation changes with the pumping of protons.
ii) One conformation has high affinity for ADP and Pi, however, when its conformation changes to that of one with high affinity for ATP, a bond is formed between ADP and Pi, generating ATP.

A conformational change in the enzyme results in a chemical change in the bound ligands.

iv) Add the appropriate step(s) to account for anerobic metabolism in yeast. What is the main purpose of this pathway (besides generating alcohol)?(2 pts) (The chemical formula for pyruvate is $CH_3COCOOH$).

Pyruvate --> acetyladehyde-->ethanol.(1/2 pt)
The main purpose of this pathway is to **regenerate NAD**+ from NADH by the reduction of acetyladehyde (recycling of electrons) .(1 1/2 pts).