Biochemistry I, Spring Term 2001 Exam 3 Solutions.

Section A: Multiple Choice

1. d
2. c
3. b ; although PPi is a high energy compound, it is not a major source of energy.
4. d was the best answer, 1 pt was given for b and c.
5. c was the best answer. 2 pts were given for b since the error was just the number of electrons. 1 pt was given for d, although FAD undergoes two electron redox reactions, FADH2 is already reduced and cannot accept any more electrons.
6. d
7. b and c are correct and 1.5 pts was given for either. Although insulin eventually causes dephosphorylation of proteins, it first activates a kinase.
8. c is the correct answer. 1 pt was given for d since the only error as the linkage number.

Part B: Short Answer

B1:i

- Keep the membranes above Tm. Above Tm the membranes are fluid.
- Change the level of unsaturated fatty acids. The kinks in the chain due to the unsaturated fatty acids will reduce the melting point.
- Add cholesterol, this will disrupt the packing of acyl chains in phospholipids, resulting in a more fluid behaviour.

ii) Biological membranes must be fluid so that proteins and electron carriers (such as coenzyme Q) can diffuse in the membrane. In addition, the conformational changes of some membrane proteins (i.e. receptors) may require a fluid membrane.

iii)

- H-bonds. Due to the absence of water in the interior of the membrane, a membrane protein must satisfy all of its H-bonds. Any h-bonds that were to water in the unfolded protein that are not formed in the membrane cost +20 kJ/mol.
- The outer surface of membrane proteins is completely non-polar (at least the part that is exposed to the acyl chains. In contrast, soluble proteins have about 50 non-polar residues on their surfaces.

B2: Since the two proteins have identical molecular weight you cannot use gel filtration to separate them. Since the isoelectric points are the same as well ion exchange (cation or anion) will not be successful as well. The identical solubility in ammonium sulfate rules out trying to ppte one protein yet leave the other in solution. The only method left is affinity chromatography. Specifically, you would need to make a column that binds one protein, but not the other. For example, a column with fructose as the ligand would likely bind aldolase but not pyruvate kinase since pyruvate kinase binds 3 carbon sugars.

B3: The steps, and associated names and cofactors are:

III
↓ Dehydrogenase, using NAD^+ or FAD, producing NADH or $FADH_2$
IV
↓ enolase
II
↓ Kinase (in this direction), requiring ATP. Phosphatase in the reverse direction.
I

The ordering of the first three compounds are the based on the initial oxidation of fatty acids or part of the TCA cycle. The last step is phosphorylation of an OH group.

As for regulation, the 1st step is likely to be regulated. This is also an energetic step, and might be regulated by NAD, etc.

B4:

i): 6 carbon ketose to a 3 carbon ketose (DHP) and a 3 carbon aldose (G-3-P)

ii):

$\Delta G = \Delta G^o + RT \ln [DHP][G3P]/[F16]$

$0 = 24 \text{ kJ/mol} + 2.5 \ln(150 \text{ uM})[G3P]/(30 \text{ uM})$

$-24 = 2.5 \ln(150 \text{ uM})[G3P]/(30 \text{ uM})$

$-9.6 = \ln(150 \text{ uM})[G3P]/(30 \text{ uM})$

$[G3P] = 13.5 \text{ uM}$

B5: Electrons are transferred through membrane protein in the mitochondrial inner membrane, for example: Complex I - CoQ - Complex 3 - cytochrome C - complex IV. During this process protons are pumped across the membrane, creating a pH gradient. ATP is synthesized from this pH gradient. The actual reaction, ADP+Pi-->ATP is driven by a conformational change in the enzyme.

B6: Fatty acids are synthesized (and degraded) two carbons at a time.

C1:

Part A: An overall sketch of the metabolic pathways was expected, similar to that shown in class. Specific points were given for the following:

- Starch: Enters glycolysis as glucose, finishing with Pyruvate, which is converted to Acetyl-CoA, producing 2ATP, 4 NADH (2 from glycolysis, 2 from pyruvate decarboxylase)
- Fats: Enter fatty acid oxidation (β-oxidation), producing 1 acetyl-CoA, 1 NADH, 1 $FADH_2$/ cycle.
- Proteins: Amino acids enter TCA cycle directly via oxaloacetate and α-ketoglutaric
- TCA cycle: Release of CO_2, from acetyl-CoA. Production of 3 NADH, FADH, 1 ATP

Part B:

i) Insulin will lead to dephosphorylation of glycogen synthase and glycogen phosphorylase. The former is activated, the latter is inactivated by this event. Therefore glycogen is made from glucose.

ii) Epinephrine will ultimately lead to the phosphorylation of glycogen synthase and glycogen phosphorylase. The former is inactivated, the latter is activated by phosphorylation. Therefore glucose is released from glycogen. It can then travel to your brain via the blood

C2:

i) Examples of different steps:
Glycolysis/gluconeogenesis:

1. Glucose -> Glucose-6-P: Requires ATP in the forward direction, spontaneous in the reverse direction.
2. Fructose-6-P --> Fructose-1,6-P. Requires ATP in the forward direction, spon. in the reverse direction.
3. PEP --> Pyr. Spontaneous as written, the reverse reaction requires the synthesis of oxaloacetate, followed by the release of CO2.

Fatty Acid Degradation:

1. Formation of Acyl-CoA: This requires two ATP equivalents in the degradative pathway. The hydrolysis of Acyl-CoA to release the newly synthesized fatty acid is spontaneous.
2. Release of Acetyl-CoA: In degradation, the shortening of the chain by the release of acetyl-CoA is spontaneous, while the addition of two new carbons to a growing chain requires activation of acetyl-CoA to malonyl-CoA, followed by release of CO2 during the addition of the two carbon unit to the growing chain.

Glycogen:

1. Synthesis requires the formation of the UDP-glucose from UTP and G-1-P. Degradation is spontaneous with the release of G-1-P.

In all cases, one direction is energetically "down hill" and proceeds spontaneously. Consequently, the other direction is "up hill" and requires energy for the reaction to proceed in that direction.

ii) In glycolysis PFK is activated by AMP/ADP and inhibited by ATP. In the TCA cycle Pyr decarboxylase, citrate synthase, a-ketoglutarate dehydrogenase and isocitrate dehydrogenase are, in general, activated by NAD^+ and inhibited by NADH. In both cases the pathways are inhibited when the cell has lots of energy (ATP/NADH) and activated when the cell needs to make energy (ADP/NAD).

iii) The key regulatory agent is citrate. High levels of citrate inhibit PFK and prevent the use of sugars as energy sources. High levels of citrate would arise on an amino acid diet because amino acids enter the TCA cycle directly.

C3: No one attempted this one. Good question for the final perhaps?