**BS11  Answer Key to Second Mid-Term  Spring 1998**

**Question 1.** (Note: these should look familiar.)

7 pt) **A.** Some microorganisms synthesize 2-deoxy-D-ribose-5-phosphate from glyceraldehyde-3-phosphate and X with one enzyme:

\[
\begin{array}{ccc}
HC=O & \\
\downarrow & \\
HC-OH & \text{+} & X \\
\downarrow & \text{ } & \\
H_2C-OP & \rightarrow & HC=O \\
\downarrow & \\
HC-OH & \text{ } & \\
\downarrow & \\
HC-OH & \text{ } & \\
\downarrow & \\
H_2C-OP & & \\
\end{array}
\]

Draw the structure of X.

**Ans:** This reaction should remind you of those catalyzed by TIM and aldolase. The compound X must contain two carbon atoms. Further, it must have a carbonyl beta to the carbon being joined to GAP to stabilize the carbanion formed during the reaction. The leaving group from X is a proton which can be added back to the carbon that was at the 1 position in GAP.

So, the structure of X is:

\[
HCCH_3
\]

5 pt) **B.** A yeast extract, containing all the enzymes required for alcoholic fermentation, is incubated anaerobically in 1 l of a medium containing: 200 mM glucose, 20 mM ADP, 40 mM ATP, 2 mM NADH, 2 mM NAD\(^+\) and 5 mM As (arsenate).

What is the maximum amount of ethanol that can be formed?

- a) 2
- b) 20
- c) 40
- d) 200
- e) 400

**Ans:** (e) 400 mmoles. Arsenate can be used by the enzyme G3P dehydrogenase in place of phosphate. However, 1-arseno-3-phosphoglycerate is unstable and will spontaneously hydrolyze to give 3-phosphoglycerate and arsenate. Since arsenate is regenerated, its concentration will not be limiting. And since the ATP will be regenerated by pyruvate kinase and the NAD\(^+\) regenerated by pyruvate decarboxylase, neither of these will be limiting either. Therefore, all of the glucose will be converted to ethanol.
5 pt) **C.** Erythrocyte ghosts (sealed plasma membranes without cytoplasmic contents) are resealed so that they contain ADP, Pi, MgCl$_2$ and 150 mM KCl. They are washed and transferred to a solution containing 150 mM NaCl. After a short time the contents of the ghosts are analyzed.

a) there is no change with time

b) ATP is present

c) there is only an increase of Na

d) there is an increase in K

**Ans:** (b) **ATP is present.** In oxidative phosphorylation, a proton gradient drives ATP synthesis. In this case, the large Na$^+$ and K$^+$ gradients are forcing the Na$^+$/K$^+$ pump to run backwards to synthesize ATP. The fact that such gradients can cause the formation of ATP was known even before Peter Mitchell proposed his chemiosmotic hypothesis.

7 pt) **D.** Mitochondria are suspended in phosphate-buffered solution containing β-OH-butyrate and O$_2$. At the indicated time, 500 nmole of ADP were added.

![Graph showing O$_2$ consumption over time]

**a) Why does O$_2$ consumption increase when ADP is added to the suspension?**

**Ans:** The rate of O$_2$ consumption is initially limited by the opposing proton gradient. When ADP is added to the suspension, the proton gradient can collapse because protons can now reenter the matrix through the ATP synthetase, and so O$_2$ consumption increases.

**b) What is the P/O ratio?** 2.5. [100 nmol of O$_2$ or 200 nmol of O for 500 nmol of P]
10 pt) E. A suspension of mitochondria is placed in a solution containing 3-OH-butyrate, O₂, ADP and KCl. The pH of the external solution changes under these conditions and attains a value of pH 6. The intramitochondrial pH is 9. When valinomycin is added to the external solution, the K⁺ concentration inside the mitochondria increases. At the steady state, the extracellular (K⁺) is 0.5 mM and the intracellular (K⁺) is 100 mM. Determine the value of the membrane potential for these mitochondria in the absence of valinomycin. (Faraday’s constant is 23 kcal/V·mol.)

**Ans:** We can determine the membrane potential associated with the proton gradient by using the information about the K⁺ equilibrium in the presence of valinomycin. The K⁺ will equilibrate in such a way that it will cancel out the membrane potential. So,

\[
(\psi_i - \psi_o) = \frac{RT}{zF} \ln \left( \frac{[K^+_{\text{out}}]}{[K^+_{\text{in}}]} \right) \\
= \frac{[(600 \text{ cal/mol})/(1)(23 \text{ kcal/V·mol})]}{\ln (0.5/100)} \\
= -140 \text{ mV}
\]
20 pt) **Question 2.** Mitochondria are suspended in buffered 0.1 M KCl containing O₂ and Pi. The amount of O₂ consumed is measured in the basal state and after the additions shown in the Figure. Each compound is added in excess and remains in place for the remainder of the experiment. Valinomycin is a K⁺ ionophore; nigericin is a K⁺/H⁺ exchanger; atracyloside is an inhibitor of the ATP/ADP exchanger. Complete the graph.

Make sure that the relative slope of each segment is clear!

*Ans:* Valinomycin alone will have no effect on the basal rate of O₂ consumption. ADP will increase the rate since protons can now flow back in through the ATP synthase. Since atracyloside keeps the ADP from entering the mitochondria, the rate will return to the basal state. When nigericin is added, the proton gradient is completely dissipated, so O₂ consumption reaches its maximal rate.

**Question 3.** N-ethylmaleimide (NEM) often inactivates polypeptides by alkylating them at free sulfhydryl groups. In an effort to assign distinct functions to the various protein components of SRP, Peter Walter's laboratory has used NEM to selectively inactivate different protein subunits of SRP.

The following experiment was performed to assess the function of SRP: 1) SRP was added to an *in vitro* translation system (from wheat germ extracts) that contained ⁵⁵S-methionine and an mRNA for a protein normally exported to the ER lumen. 2) A portion of the mixture created in step 1 was then added to purified microsomes. 3) After incubation for 5 minutes, a portion of each mixture was then incubated with or without a non-specific protease. 4) The resulting radioactive polypeptides were then analyzed by SDS-PAGE and autoradiography.
This protocol was performed using unmodified SRP; SRP containing alkylated forms of the 9 and 14 kDa subunits; SRP containing alkylated forms of the 68 and 72 kDa subunits; or SRP containing alkylated forms of the 9, 14, 68 and 72 kDa subunits. The results are shown below.

<table>
<thead>
<tr>
<th>Protease</th>
<th>SRP + in vitro translation system</th>
<th>SRP + in vitro translation system + microsomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>unmodified SRP</td>
<td>unmodified SRP</td>
</tr>
<tr>
<td></td>
<td>SRP 9/14 alkylated</td>
<td>SRP 9/14, 68/72 alkylated</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>SRP 68/72 alkylated</td>
<td>SRP 9/14, 68/72 alkylated</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
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<tr>
<td></td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

5 pt) A. From this data, what is the most likely function of the 9 and 14 kDa polypeptides? Be brief.

**Ans:** In the absence of microsomes, the unmodified SRP lane shows no band in the absence of protease, but the SRP 9/14 alkylated lane does, so the 9 and 14 kDa polypeptides must be involved in **translation arrest**.

5 pt) B. 1. What is a reasonable explanation for why NO bands are present in the SRP 68/72 alkylated lane in the presence of microsomes?

**Ans:** Since in the absence of microsomes, the SRP 68/72 alkylated lane shows no band, the 68 and 72 kDa proteins must not be involved in translation arrest; translation arrest occurs normally. However, SRP with 68/72 alkylated must be defective in the relief of translation arrest prior to translocation. This can be concluded from the lack of any bands in the SRP 68/72 alkylated lane in the presence of microsomes and from the presence of a large, unprocessed, protease sensitive band in the SRP 9/14 and 68/72 alkylated lane which never arrested but did not get translocated.

5 pt) 2. What is a possible function that the 68 and 72 kDa proteins could be performing?

**Ans:** The 68 and 72 kDa proteins could be involved, for example, in interacting with the SRP receptor.

5 pt) C. What do these results imply about the importance of translational arrest with respect to translocation into the ER lumen? Be brief.

**Ans:** In the presence of microsomes, the SRP 9/14 alkylated lane shows the same smaller MW protected, processed band as seen for the unmodified SRP. This indicates that, in spite of the lack of translation arrest, most of the protein was translocated into the microsomes. Therefore, in this case, translation arrest is not necessary for translocation.
Question 4. Ubiquinol cytochrome c oxido-reductase (Complex III) has been purified from beef heart mitochondria and reconstituted into proteoliposomes together with succinate dehydrogenase (Complex II) to yield vesicles that have succinate-cytochrome c reductase activity.

The reconstitution system contained 1.5 nmoles succinate dehydrogenase, 1 nmole ubiquinol cytochrome c oxido-reductase, 10 mg egg phospholipids, 10 mg deoxycholate (detergent), and 10 μmoles succinate in 1 ml of 50 mM phosphate buffer. After removal of the detergent by dialysis, the sealed vesicles (in 1 ml of solution) were placed in a titration vessel that allows measurement of the pH of the solution. After addition of 0.1 mM ubiquinol/ubiquinone and 1 μM cytochrome c, the pH was adjusted to 7. The reaction was started by adding 5 nmol of ferricyanide (Fe(CN)$_6^{3-}$) to the solution. In a second experiment, 5 μM dinitrophenol (DNP, a proton ionophore) was added before the addition of ferricyanide.

The following results were obtained:

<table>
<thead>
<tr>
<th>pH</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>+0.03</td>
<td>+0.25</td>
</tr>
</tbody>
</table>

Faraday’s constant is 23 kcal/V·mol and the standard reduction potentials for the following half cells are:

<table>
<thead>
<tr>
<th>Half Cell</th>
<th>$E'_0$ (volt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fumarate/Succinate</td>
<td>+0.03</td>
</tr>
<tr>
<td>Cyt c (ox)/Cyt c (red)</td>
<td>+0.25</td>
</tr>
<tr>
<td>Ferricyanide (+3)/Ferrocyanide (+2)</td>
<td>+0.36</td>
</tr>
</tbody>
</table>
5 pt) A. Draw a cartoon showing the flow of electrons through the relevant complexes and compounds in this reaction. (You don't need to include the specific e' carriers internal to the complexes.) Indicate the inside and outside of the vesicle.

**Ans:**

![Diagram of electron flow through complexes II and III.](image)

Complex: II III

5 pt) B. Write the complete equation that describes the overall reaction between succinate and ferricyanide. Write out the structures of the compounds.

**Ans:**

\[
\text{COO}^- + 2 \text{Fe(III)(CN)}_6^{3-} \rightleftharpoons \text{CH}_2 + 2 \text{H}^+ + 2 \text{Fe(II)(CN)}_6^{4-}
\]

5 pt) C. What is the value of \( \Delta G^{\circ'} \) for this reaction.

**Ans:** \( \Delta G^{\circ'} = -nF\Delta E_0' = -2 \times (23 \text{kcal/V mol}) \times (0.36 \text{ V} - (0.03 \text{ V})) = -15 \text{kcal/mol} \)

5 pt) D. From the graph, what is the value of \( \text{H}^+/2e^- \) in the absence and the presence of DNP.

**Ans:** In the absence of DNP, the total change in \( \text{H}^+ \) is about 10 nmole for the 5 nmole of \( \text{Fe(CN)}_6^{3-} \) added. Since each \( \text{Fe(CN)}_6^{3-} \) only accepts one electron, the \( \text{H}^+/2e^- \) ratio is therefore 4. In the presence of DNP, the change in \( \text{H}^+ \) is about 5 nmole, so the \( \text{H}^+/2e^- \) ratio is only 2.

5 pt) E. Explain the effect of DNP on the value of \( \text{H}^+/2e^- \).

**Ans:** The key here is to realize that the conversion of succinate to fumarate passes two protons to CoQ which are subsequently released into the solution (see diagram in (A)). Even though DNP causes the protons pumped by complex III to immediately re-equilibrate back inside the vesicles, DNP cannot change the fact that there are more protons in the system. Even though DNP will cause these two released protons to equilibrate with the inside of the vesicles, the volume inside the vesicles is small relative to the whole. (In the absence of DNP, over time, the change in pH returns to the level seen with DNP indicating that the protons pumped out are able to leak back into the vesicles.)