BIO 451
23rd October, 2002

KEY

This exam may be taken apart for grading. Please PRINT your name on each page.
If you do not have sufficient room for your answer in the space provided, please continue on the back of the page on which the question appears.

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Total 100

NOTE: FULL CREDIT WILL BE GIVEN FOR THOSE ANSWERS THAT CLEARLY ADDRESS ALL RELEVANT ASPECTS OF THE QUESTIONS IN THE CLEAREST AND MOST CONCISE MANNER..
I. [20 points; 2 points for each correct responses possible. 1 point off for each incorrect guess]
For each of the items listed below two statements are provided. Some of the statements are true and some of them are false. Identify, by a check mark the ONE completely TRUE statement for each part. If you mark two choices, 2 points will be deleted for the incorrect extra choice.

---[]---- The consensus sequence for N-linked sugars is --Asn-X-Ser-- or --Asn-X-Thr--.

A. With respect to glycosylation of membrane constituents:

------ Glycosylation occurs exclusively on peripheral membrane proteins on the cytoplasmic face of the plasma membrane.

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------ catalyzes hydrolysis of the pyrophosphate linkage between the [] and [] phosphates of ATP.

B. Inorganic pyrophosphatase

---[]--- catalyzes a reaction that provides the driving force for group activation in many biosynthetic reactions.

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---[]--- leads to inability to effectively metabolize galactose

This Q should have been edited; as written either answer was accepted.

C. Genetic deficiency in galactose-1-phosphate uridylyl transferase.

---[]---- leads to cataract formation due to accumulation of galactitol

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------ contain at least one copy of the consensus sequence for attachment of –linked sugars [Asn-X-Ser or Asn-X-Thr].

D. Many putative membrane-spanning regions of polypeptides will:

----[]-- typically are at least 20 residues in length.

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E. The glycolytic pathway requires which three of the following ‘irreversible’ regulatory enzymes?

----□---- Hexokinase, pyruvate kinase, and PFK

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F. Cleavage of F-1,6-bisP, catalyzed by the action of aldolase, followed by the action of triosephosphate isomerase, leads to the equivalency of which pairs of carbons of glucose with respect to their appearance in pyruvate?

----□---- Carbons 1 and 6, 2 and 5, 3 and 4

-------------

G. Sialoglycoproteins bind to which of these lectins

----□---- Wheat germ agglutinin

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----□---- Pyruvate dehydrogenase

H. Catalyzes the committed step in the citric acid cycle

----□---- Citrate synthase

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----□---- Succinate dehydrogenase

I. The only enzyme of the citric acid cycle that is membrane-bound.

------ □-ketoglutarate dehydrogenase

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------ □---- Succinyl-CoA synthetase

J. The only enzyme of the citric acid cycle that catalyzes substrate level phosphorylation

------ Citrate synthase

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The figure on the next page represents a segment of a glycogen molecule; the structure of the glucose residues is simplified.

**A.** What single feature is most incorrect in this figure? (2 pts)

*The branch points are too close together, they are typically every 8-12 residues.*

**B.** Identify (circle and label) the residues that would be susceptible to the action of glycogen phosphorylase. (2 pts)

*Starting on the left (non-reducing) end of the figure, phosphorylase would cleave one glucose at a time up to about four residues from the branch point.*

**C.** Identify (circle and label) the residues that would be subject to the action of debranching enzyme (after phosphorylase action). (2 pts)

*Three of the four residues PLUS the fourth (the glucose linked by 1,6).*

**D.** Identify (mark and label) the residue(s) to which glycogenin would be attached. (2 pts)

*The single residue on the reducing end (far right).*
III. [10 points]

The $K'_{eq}$ of the adenylate cyclase reaction ($ATP \equiv cAMP + PP_i$) was found to be 0.065. If the $G'$ for ATP hydrolysis to 5'-AMP + PP_i is taken to be -33.44 kiloJoules/mole, calculate the $G'$ for the conversion of cAMP to 5'-AMP. \left[ R = 8.314 \text{Joules per degree Kelvin per mol} \right]

You must show ALL your rationale and calculations.

\[
cAMP + PP_i \rightarrow ATP \quad K'_{eq} = \frac{1}{0.065} = 15.38
\]

\[
G = -2.303 (8.314 \text{J/deg/mol}) (298 \text{deg}) \log 15.38 = -6.77 \text{kJ/mol}
\]

\[
ATP \rightarrow 5'AMP + PP_i \quad -33.44 \text{kJ/mol}
\]

\[
cAMP + PP_i \rightarrow ATP \quad -6.77 \text{kJ/mol}
\]

\[
cAMP \rightarrow 5'AMP \quad -40.21 \text{kJ/mol}
\]
IV. [6 points] Answer A OR B but not both. Only your first answer will be graded]

A. Describe the function and physiological role of either the anion carrier or the Na⁺K⁺ ATPase in as much detail as you are able. NOTE You are NOT being asked to describe the mechanism of either one of them.

*Please see the text and Handout #6.*

B. 1. Use the grid on the next page to generate a hydropathy plot for putative integral membrane protein comprised of 150 residues, having two helical membrane spanning domains. Assume a minimum cutoff of +1. Explain your rationale and the basis for such analyses in as much detail as you are able. (3 pts)

*A complete answer will be an irregular wavy line that covers the entire sequence, with only two peaks that rise well above +1, covering ~20 residues. There should also be a statement as to why the hydropathy index is positive, and why the helical membrane spanning region includes ~20 residues. Please award partial credit if justified.*

2. How might you assess (primary structure only) whether the protein may be N-glycosylated? Explain the rationale. (3 pts)

*Look for the consensus sequence --- see part A of QI.*
V. [5 points]  
In their classic studies of fermentation of glucose, Harden and Young (1906) found that when limited amounts of phosphate were added to a yeast extract fermentation ceased before all of the glucose was consumed. Explain.

*Pi is a required substrate for glycolysis.*

VI. [10 points]  
Identify and state the metabolic/biochemical significance of the items listed below; your answers must be consistent with material covered in class or assigned in the text. **Choose FIVE; only the first five answers will be graded.** PLEASE ANSWER ON THE BACK OF THIS PAGE, NOT IN THE NARROW SPACE BESIDE EACH ITEM.

a) Glycogenin  
b) HMG-CoA  
c) UDP-glucose  
d) Branching enzyme  
e) EndoH  
f) Proteinase K  
g) Iron-Sulfur cluster  
h) Calmodulin  
i) PrP 27-30  
j) Protein only hypothesis  
k) [-adrenergic receptor  
l) FBPase 2  
m) cAMP-dependent protein kinase  
n) asialoglycoprotein receptor  
o) Concanavalin A  
p) Valinomycin  
q) Porin (not aquaporin)  
r) 6-Phosphogluconolactone  
s) UDP-galactose  
t) [-secretase  

*Please see the text or handouts.*
VII. (8 points) **Answer A OR B but not both. Only your first answer will be graded.**

A.
1. On 4 October I summarized a current hypothesis regarding the normal function of \( \text{PrP}^C \). State (briefly) this postulated role. (4 points)

*It is postulated to be involved in the transport of copper ions.*

2. If this hypothesis is correct, this function might be inactivated by diethylpyrocarbonate. Why? (4 points)

*Transport of copper ions is thought to be mediated by formation of chelates by multiple imidazole groups of H residues in the N-terminal portion of PrP. The reagent covalently modifies imidazole groups.*

B.
An investigator isolated a microorganism from an unusual environment. She was able demonstrate that the citric acid cycle enzymes were operative but some preliminary labeling experiments suggested that the aconotase in this organism might exhibit a selectivity in the conversion of citrate to isocitrate that is different from that found in most organisms. Following radiolabeling of oxaloacetate, and allowing the citric acid cycle to proceed in this organism for a short time, radiolabeled \( \alpha \)-ketoglutarate was isolated. The two possible labeling patterns are illustrated in the figures below.

1. Which enzyme of the citric acid cycle would be most useful in determining the labeling pattern in the isolated \( \alpha \)-ketoglutarate. Explain (3 pts)
-ketoglutarate dehydrogenase will catalyze the decarboxylation of the carboxyl group adjacent to the carbonyl group.

2. Identify the five cofactors that are required for this enzyme. (5 pts)

*CoA, ThPP, Lipoamide, NAD, FAD*

VIII. (4 points)

Analysis (by scanning calorimetry) of the effect of temperature on the structure of purified phospholipids (in liposomes) demonstrates sharp transitions at elevated temperature; the temperature at which these transitions occur is dependent on chain length and degree of saturation of the constituent fatty acids.

Given the similarity between the bilayers of lipids in liposomes and the lipid bilayers of plasma membranes, why is it that naturally occurring membranes do not exhibit sharp transitions as a function of temperature, although it is clear that the structure of the membranes is altered?

*Biological membranes are heterogeneous, whereas liposomes typically may be comprised of a single or few phospholipids. In addition membranes contain proteins as well. It is analogous to melting point determinations; impure compounds tend to melt over a wide range.*
IX. [ 9 points]
Many mitochondrial proteins are synthesized in the cytosol. This question asks you to recall the mechanism by which these proteins are translocated from the cytosol into the mitochondrial matrix and to focus on the role of protein conformation in this process. For EACH part, your answer must specifically relate the observation to the role of protein conformation.

A.
1. Describe the effect of prior treatment of a protein with urea on the rate of its translocation into the mitochondrion. (2 pts)

**Proteins must be at least partially unfolded in order to be translocated from the the cytosol to the mitochondrion. Urea is a chaotropic agent that tends to unfold proteins. Prior treatment with urea enhances the rate of translocation.**

2. Genetically engineered shutdown of Hsp70 production in yeast causes these cells to accumulate precursor forms of proteins that are otherwise imported into mitochondria. Explain. (3 pts)

**The role of Hsp70 is to facilitate the movement of proteins across the two mitochondrial membranes; partial unfolding is part of the mechanism. Thus, in the absence of this chaperone, proteins destined to move into the mitochondrion will accumulate in the cytosol.**

B.

Dihydrofolate reductase (DHFR) a normally cytosolic enzyme, is imported into yeast mitochondria when the protein is engineered to include the targeting sequence of a cytosolically synthesized mitochondrial protein.

1. The importation of DHFR is inhibited by the addition of methotrexate, an analog of the normal substrate of DHFR. The translocation of other proteins (ones that do not bind methotrexate) to the mitochondrion is not inhibited by addition of methotrexate. (4 pts)

**The binding of methotrexate will stabilize the folded (compact) form of DHFR; thus blocking translocation. Those who said that the target recognition sequence becomes inaccessible were given partial credit; depending on the overall context of the answer provided.**
X. [10 points] In order to answer this question you need to be familiar with the details of the isozyme-specific allosteric regulation of glycogen phosphorylase by 5'-AMP and glucose, respectively. Describe the biochemical and physiological consequences of a defect in the binding sites for 5'-AMP and glucose for their respective tissue-specific phosphorylases. News of which of these defects would be most devastating to a patient? Explain.

5'-AMP activates muscle phosphorylase b; it "kick starts" the system. The major activation of phosphorylase is mediated by covalent modification $[b \rightarrow a]$. This is totally independent of 5'-AMP.

Glucose is the major allosteric regulator of liver phosphorylase. This normally is mediated by a conformational change that makes the P's on Ser in phosphorylase a accessible to PP1, thus inactivating it $[a \rightarrow b]$; this leads to release of PP1 and subsequent removal of P from glycogen synthase; this activates it.

If the glucose binding site on phosphorylase a is defective, phosphorylase a would remain "on" (continually degrading glycogen) and glycogen synthase would remain "off".

Given the major role of the liver in homeostasis, this defect would be far more serious than a defect in allosteric activation of muscle phosphorylase by 5'-AMP.
XI. [10 points]

A. How are the function and processing of CFTR assessed; i.e., what types of analyses are performed? (6 points)

*Function is assessed by measuring cAMP-dependent chloride ion transport.*

*Processing is assessed by SDS-PAGE; one looks for the mature (high MW) fully glycosylated species.*

B. In the context of the material presented in Handout 7, to what does F508 refer? (2 points)

*It refers to a deletion of a phenylalanine residue at position 508 in the CFTR protein.*

C. What is the significance of F508? (2 points)

*It represents the most common defect leading to cystic fibrosis.*
B. An investigator isolated a microorganism from an unusual environment. She was able to demonstrate that the citric acid cycle enzymes were operative but some preliminary labeling experiments suggested that the aconitase in this organism might exhibit a selectivity in the conversion of citrate to isocitrate that is different from that found in most organisms. Following radiolabeling of oxaloacetate, and allowing the citric acid cycle to proceed in this organism for a short time, radiolabeled $\alpha$-ketoglutarate was isolated. The two possible labeling patterns are illustrated in the figures below.

![Chemical structures](attachment:image.png)

1. Which enzyme of the citric acid cycle would be most useful in determining the labeling pattern in the isolated $\alpha$-ketoglutarate? Explain (3 pts)
   - $\alpha$-Ketoglutarate dehydrogenase, it will catalyze the oxidative deamination of the carbonyl adjacent to the carbonyl. $a$ will yield radiolabeled CO$_2$ and $b$ will not.

2. Identify the five cofactors that are required for this enzyme. (5 pts)
   - Coenzyme A
   - Thiamine pyrophosphate
   - Lipoic acid
   - NAD
   - FAD
branching enzyme → free glucose

Branching enzyme acts on glycogen to produce free glucose and glycogen phosphorylase.

Typically, branch points are every 8-12 glucose residues.
Positive values of hydrophy index reflect the energy associated with removal of a.a. side chains from a hydrophobic environment in the lipid bilayer and placing them in water. The translation along the helix per residue corresponds to ~15Å.

1.5Å x 20 residues = 30Å ≈ thickness of the hydrophobic bilayer.