Chem 465
Biochemistry II

Multiple choice (4 points apiece):

1. Which of the following statements is not true concerning glycolysis in anaerobic muscle?
   A) Fructose 1,6-bisphosphatase is one of the enzymes of the pathway.
   B) It is an endergonic process.
   C) It results in net synthesis of ATP.
   D) It results in synthesis of NADH.
   E) Its rate is slowed by a high [ATP]/[ADP] ratio.
   B is the book answer, but I accepted A as well!

2. Glucose labeled with 14C in C-1 and C-6 gives rise in glycolysis to pyruvate labeled in:
   A) A and C.
   B) all three carbons.
   C) its carbonyl carbon.
   D) its carboxyl carbon.
   E) its methyl carbon.

3. The main function of the pentose phosphate pathway is to:
   A) give the cell an alternative pathway should glycolysis fail.
   B) provide a mechanism for the utilization of the carbon skeletons of excess amino acids.
   C) supply energy.
   D) supply NADH.
   E) supply pentoses and NADPH.

4. Which one of the following types of mechanisms is not known to play a role in the reversible alteration of enzyme activity?
   A) Activation by cleavage of an inactive zymogen
   B) Allosteric response to a regulatory molecule
   C) Alteration of the synthesis or degradation rate of an enzyme
   D) Covalent modification of the enzyme
   E) Interactions between catalytic and regulatory subunits

5. Which combination of cofactors is involved in the conversion of pyruvate to acetyl-CoA?
   A) Biotin, FAD, and TPP
   B) Biotin, NAD+, and FAD
   C) NAD+, biotin, and TPP
   D) Pyridoxal phosphate, FAD, and lipoic acid
   E) TPP, lipoic acid, and NAD+

6. The oxidative decarboxylation of -ketoglutarate proceeds by means of multistep reactions in which all but one of the following cofactors are required. Which one is not required?
   A) ATP
   B) Coenzyme A
   C) Lipoic acid
   D) NAD+
   E) Thiamine pyrophosphate
7. The conversion of 1 mol of pyruvate to 3 mol of CO₂ via pyruvate dehydrogenase and the citric acid cycle also yields _____ mol of NADH, _____ mol of FADH₂, and _____ mol of ATP (or GTP).
   A) 2; 2; 2
   B) 3; 1; 1
   C) 3; 2; 0
   D) 4; 1; 1
   E) 4; 2; 1

Longer questions - 15 points each - Choose any 4!

8. Here is a list of several cofactors or substrates that are used in enzymes in either glycolysis, the TCA cycle or the anapleotropic reactions. For each cofactor or substrate, name at least one enzyme in which it is used, and what it’s function is in that reaction. (If you can’t remember the name of the enzyme, I will accept the structure of the reactant and product in the reaction) Mg²⁺
   Hexokinase, Phosphohexose isomerase, Phosphofructokinase, phosphoglycerate kinase, Phosphoglycerate mutase, enolase or pyruvate kinase. Used to help bind ATP or ADP.

   Coenzyme A
   Pyruvate dehydrogenase, or α-ketoglutarate dehydrogenase. Carries acetate

   NAD⁺
   Pyruvate dehydrogenase, glyceraldehyde 3-Phosphate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, L-malate dehydrogenase. Water soluble electron carrier

   Lipopoate
   Pyruvate dehydrogenase, or α-ketoglutarate dehydrogenase. Carries the hydroxyethyl created from E1 to where it can be transferred to CoA. Then reduces FAD.

   FAD
   Pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, or Succinate dehydrogenase. Lipid soluble electron carrier

   TPP
   Pyruvate dehydrogenase, or α-ketoglutarate dehydrogenase. Makes the carbon attached to it more reactive.

   Fe
   Aconitase, succinate dehydrogenase. Used in oxidation reduction reactions.

   Mn
   Pyruvate kinase part of mechanism

   Biotin
   Pyruvate carboxylase Binds HCO₃⁻

   GDP
   Succinyl-CoA synthetase. Alternate to ADP
9. This page is blank because I want you to fill it in with the glycolytic pathway from glucose to pyruvate by showing the structure of all intermediates. At each step also give the name of the enzyme and $\Delta G$ of the reaction. To help you on your way, here is a list of all then enzymes in alphabetical order: Aldolase, Enolase, Glyceraldehyde 3-phosphate dehydrogenase, Hexokinase, Phosphofructokinase-1, Phosphoglycerate kinase, Phosphoglycerate mutase, Phosphohexose isomerase, Pyruvate kinase, Triose phosphate isomerase.

On this key I am not going to take the time to make the structures, but I will check that you have the correct structure

Glucose + ATP

\[
\text{Hexokinase} \quad \text{Mg}^{2+} \quad -16.7
\]

Glucose-6-P

\[
\text{Phosphohexose isomerase} \quad \text{Mg}^{2+} \quad +1.7
\]

Fructose-6-P + ATP

\[
\text{Phosphofructokinase-1} \quad \text{Mg}^{2+} \quad -14.2
\]

Fructose 1,6-bisphosphate

\[
\text{aldolase} \quad +23.8
\]

Dihydroxyacetone phosphate

\[
\text{Glyceraldehyde 3-phosphate isomerase} \quad +7.5
\]

Glyceraldehyde 3-phosphate + NAD

\[
\text{glyceraldehyde 3-phosphate dehydrogenase} \quad +6.3
\]

1,3-Bisphosphoglycerate

\[
\text{phosphoglycerate kinase} \quad \text{Mg}^{2+} \quad -18.5
\]

3-Phosphoglycerate + ATP

\[
\text{Phosphoglycerate mutase} \quad \text{Mg}^{2+} \quad +4.4
\]

2-Phosphoglycerate

\[
\text{Enolase} \quad \text{Mg}^{2+} \quad +7.5
\]

Phosphoenolpyruvate

\[
\text{Pyruvate kinase} \quad \text{K}^+, \text{Mg}^{2+} \text{ or Mn}^{2+} \quad -31.4
\]

Pyruvate + ATP
10. In class we talked about how oxaloacetate and α-ketoglutarate can be directly converted to the amino acids aspartate and glutamate, respectively, using a simple transamination reaction.

A. What intermediate in the glycolytic pathway can be converted to alanine using the same kind of transamination reaction?

B. If this amino acid is labeled in the carboxyl acid with C-14, and this amino acid is then feed into the glycolytic pathway using the reverse of the above reaction, will the C-14 appear in citric acid on the first turn of the TCA cycle? And, if so, at what position of the citric acid.

C. If this amino acid is labeled in the carboxyl acid with C-14, and this amino acid is then feed into the gluconeogenesis pathway using the reverse of the above reaction, at what position in glucose will the C-14 appear?

A. Pyruvate can be converted to alanine via transamination

B. \( \text{NH}_2-\text{CHCH}_3 \text{ COOH} \)
   
   \( \text{CH}_3\text{COOOH} \)
   
   This carboxylic acid gets removed as pyruvate is converted to Acetyl Co A
   
   So the citric acid is NOT labelled.

C. \( \text{NH}_2-\text{CHCH}_3 \text{ COOH} \)
   
   \( \text{CH}_3\text{COOOH} \)
   
   \( \text{CH}_3\text{COPO}_3\text{COOH} \) (PEP)
   
   Goes to 3 & 4 labeled Glucose
One of the key control points in both glycolysis and gluconeogenesis is the reaction of fructose-6-phosphate to fructose 1,6-bisphosphate on the glycolytic side and the reverse reaction on the gluconeogenesis side. Discuss all the ways these two reactions are controlled in your body.

Start with the simple direct allosteric controls

The two enzymes we are looking at are Phosphofructokinase-1 (PFK-1) on the glycolytic side and Fructose 1,6-bisphosphate-1 (FBP-1) on the gluconeogenesis side. PFK-1 is directly stimulated by ADP and AMP and inhibited by ATP and citrate. Conversely FBPase-1 is inhibited by AMP.

A second level of control is through the hormones insulin and glucagon. Here insulin is released to lower the blood sugar level; so we want to increase glycolysis to use up sugar. Glucagon is released when the blood sugar is too low and we want to release the blood sugar, so we want to shut down glycolysis and increase gluoneogenesis. Both of these interactions are modulated through Fructose 2,6- bisphosphate (F26BP). Let’s see how this works in a thumb nail sketch.

Glucagon ↑ a signal that blood sugar is low, liver should stop using and release into blood
Glucagon binds to a surface receptor on the liver cell, and this activates adenyl cyclase in the liver cell to make cAMP
as [cAMP]↑, the activity of cAMP kinase activity ↑
the PFK-2/FBPase-2 enzyme gets phosphorylated
PFK-2 activity lost, FBPase-2 activity gained
F26BP gets dephosphorylated so [F26BP]↓
As [F26BP]↓, PFK-1 activity↓, & FBPane-1activity ↑ so glycolysis ↓ and neogenesis ↑
so sugar build up in liver cell

Insulin ↑ A signal that the blood sugar level is too high
Insulin binding to a cell surface receptor starts a series of phosphorylation events that we skipped over in chapter 12. Included in these event is the phosphorylation of phosphoprotein phosphatase that dephosphorylates the PFK-2/FBPase-2 enzyme
As the PFK-2/FBP-2 is dephosphorylated
PFK-2 activity gained, FBPase-2 activity lost
[F26BP]↑
As [F26BP]↑, PFK-1 activity ↑, & FBPane-1 activity ↓ so glycolysis ↓ and neogenesis ↓
and glucose ↓

Another non-hormonal control thru F26BP
as [glucose]↑ flow of sugars through the pentose pathway ↑ and, in particular [xylulose-5-P]↑
this increases activity of phosphoprotein phosphatase 2A
this also will dephosphylate PFK-2/FBPase-2 enzyme
PFK-2 activity gained, FBPase-2 activity lost
[F26BP]↑
As [F26BP]↑, PFK-1↑, & FBPane-1↓ so glycolysis ↑ and neogenesis ↓ and glucose ↓
12. Sketch the pathway in which pyruvate is completely oxidized to CO₂. In this pathway be sure to identify all reactions that generate ATP, GTP, NADH or FADH₂. Also show the anapleotropic reaction that are used to fill in TCA intermediates when they are depleted.

I would prefer structures for all intermediates, and a cycle, but this is something I can put in with a word processor

Pyruvate
  pyruvate dehydrogenase CoASH,NAD, TPP,Lipoate, FAD  -33.3
Acetyl CoA
  citrate syntase -32.2
Citrate
  aconitase Iron-sulfur center 13.3
Isocitrinate + NAD →NADH
  isocitrate dehydrogenase Mn²⁺ NAD  Lost in text!
α-ketoglutarate + NAD →NADH
  α-ketoglutarate dehydrogenase CoASH,NAD, TPP,Lipoate, FAD  -33.5
Succinyl CoA + GDP or ADP →GTP or ATP
  Succinyl-CoA synthetase -2.9
Succinate + FAD →FADH₂
  Succinate dehydrogenase FAD, FeS clusters 0
Fumarate
  fumarase -3.8
L-malate + NAD →NADH
  l-malate dehydrogenase 29.7
Oxaloacetate

Anapleotropic:
  Pyruvate + HCO₃⁻ + ATP →Oxaloacetate + ADP + Pi  pyruvate carboxylase
  PEP + CO₂ + GDP → oxaloacetate + GTP  PEP carboxykinase
  PEP + HCO₃⁻ →oxaloacetate + Pi  PEP carboxylase
  Pyruvate + HCO₃⁻ + NADH→ malate + NAD  malic enzyme