Protein preamble (9 points)

Look, another license plate! I made this one on the CA DMV web site, that lets you see what an idea for a vanity plate looks like. Think I'll pass on this one. But just like I promised, here are a few questions on the one letter code and amino acid properties.

1 (2pts) Write the sequence of the peptide spelled out in the plate, using the three letter code (eg. a tripeptide of tryptophan would be TrpTrpTrp).

MetGluThrCysHisGluMet

2) How many of the R groups in the above peptide have a nitrogen molecule? ____1____

3) How many atoms of sulfur are there in this particular peptide? ______3____

4) One of the amino acids in the plate above is also found in the chymotrypsin catalytic triad we have discussed. Which one is it ____H or His or histidine_______

5) Briefly describe the function of that amino acid in the catalytic triad (one sentence)

Histidine accepts and donates protons as needed in the catalytic cycle. It participates in acid-base catalysis

6) Serine (S) is another catalytic triad amino acid. What is the function of serine in the catalytic triad (one sentence)

Serine has an OH group that performs a nucleophilic attack on the peptide bond

7 (2pts) Which amino acid on the license plate is most similar to serine, and why?

Two different answers are each acceptable:

Thr, T, or threonine, because it has an OH group like serine

or

Cys, C, or cysteine, because it has an SH group analogous to serine’s OH
Enzyme kinetics 101 (or maybe 102…) (13 points)

You study an enzyme in the laboratory. By your work, you find out that this enzyme obeys Michaelis-Menton kinetics, and so two constants, which you call A and B, describe the enzyme's behavior at all substrate concentrations, in the equation shown.

\[ V = \frac{B[E]_{tot}S}{A + S} \]

8) What is the name of curve that describes your enzymes rate as a function of substrate?

**rectangular hyperbola**

9 (2pts) Usually the constants in your rate equation have more common labels. What are they?

A is known as \( K_m \)

B is known as \( k_{cat} \)

10) What is the concentration of substrate at which your enzyme is operating at 50% of its maximum? (expressed in terms of one of the constants above).

\( S = K_m \) (or \( S = A \))

11) What is the concentration of substrate at which your enzyme is operating at 90% of its maximum? (expressed in terms of one of the constants above)

\( S = 9K_m \) (or \( S = 9A \))

12 (2pts) What is the physical meaning of B in terms of the enzyme's function?

B, which is \( k_{cat} \), is the number of reactions the enzyme performs per second at its maximum velocity

13 (4pts) Use the axes to the right to draw a Lineweaver-Burke plot for this enzyme. Label the axes, and the two intercepts. You don't need to say what the slope is.

(The y-intercept is \( 1/B[E]_{tot} \), or \( 1/V_{max} \))

14) You isolate a variant of your enzyme from another tissue, that catalyzes the same reaction, but it no longer shows Michaelis-Menton behavior. Instead, it show cooperativity. Sketch (no need for labels) what the S vs. V plot on the axes provided (with the V and S) below.

15) What is one structure feature of your new cooperative enzyme that is indicated by its rate behavior?

**The cooperative enzyme will have quaternary structure; multiple subunits**
An ATP-driven reaction: big man, little dog… (8 points)

The reaction shows an ester being formed from a carboxylic acid and an alcohol, from your organic chemistry days. Formation of this ester is energetically unfavorable; it will not happen without added energy. However, when this reaction is coupled to ATP hydrolysis, the reaction occurs spontaneously, and is catalyzed by an enzyme in the cell. The entire coupled reaction goes like this

\[
\begin{align*}
    \text{R}_1\text{C}^{-}\text{O}^{-} + \text{R}_2\text{OH} + \text{ATP} & \rightarrow \text{R}_1\text{C-O-R}_2 + \text{ADP} + \text{P}_i \\
\end{align*}
\]

We are going to explore how ATP provides the energy to form this ester. It is just like several other examples we have discussed in class. It turns out that an intermediate molecule is formed that has, you guessed it, a good leaving group, allowing the esterification reaction to occur spontaneously.

16 (2pts) Draw that intermediate molecule in the space provided. Wait! Look at the products of the reaction above to decide what your good leaving group must be. Then, think about which of the two organic reactants (not the ATP) would be the nucleophile that will bump off the good leaving group. Then draw your intermediate molecule.

17) Suppose in these conditions the ester formation reaction has a \(\Delta G\) of 26 kJ/mole, and the ATP hydrolysis has a \(\Delta G\) of -56 kJ/mole. What is \(\Delta G\) for the coupled reaction written above? \(-30\text{ kJ/mole}\)

18) What is the \(\Delta G\) for the simple hydrolysis of the ester (back into the carboxylic acid and alcohol) without ATP in these conditions? \(-26\text{kJ/mole}\)

19) An enzyme is needed for the reaction to occur as well. Does the enzyme affect the \(\Delta G\) of the coupled reaction? Yes or no, and why.

No, enzymes alter activation energy, not the free energy change of reactions; they are catalysts.

20 (3pts) Sketch an energy diagram for the coupled reaction, with and without enzyme. Label axes, indicate the \(\Delta G\) for the reaction, the activation energy \(\Delta G^\dagger\), and the effect of the enzyme. You can use the abbreviations below to make the drawing less cluttered.

\[
\begin{align*}
    \text{R}_1 + \text{R}_2 + \text{ATP} & \rightarrow \text{P} + \text{ADP} + \text{P}_i \\
\end{align*}
\]

\[
\begin{align*}
    \text{R}_1\text{C}^{-}\text{O}^{-} & \rightarrow \text{P} + \text{ADP} + \text{P}_i \\
\end{align*}
\]
An inborn error in pyruvate dehydrogenase (11 points)

The pyruvate dehydrogenase (PDH) complex is a key enzyme complex that connects glycolysis to subsequent oxidation of glucose. A number of cases have been reported where a patient has a deficiency in PDH activity due to a genetic loss of one or more activities of the complex. Understandably, the symptoms of PDH deficiency (PDHD) are severe, often resulting in infant mortality, or survival to adulthood with extreme neurological symptoms.

21 (2pts) Write the balanced reaction for what PDH does. You do not need to include cofactors (we'll get to them below), just all reactants and products. No structures needed here.

\[
\text{Pyruvate} + \text{HS-CoA} + \text{NAD}^+ \rightarrow \text{Ac-CoA} + \text{NADH} + \text{CO}_2 + \text{H}^+ 
\]

22 (2pts) The four cofactors NAD, FAD, TPP, lipoic acid all function in PDH catalysis. Write the order of involvement of the cofactors in the PDH reaction as you wrote it above. Just the order, nothing fancy. Like this: NAD, FAD, TPP, lipoic acid. Or whatever.

ORDER: TPP, lipoic acid, FAD, NAD

23 (3pts) Which of the four PDH cofactors are represented below? Label each, and use "none" if the picture is not associated with any of them.

**TPP**

**lipoic acid**

**none (biotin)**

24) Patients with a severe PDH deficiency have limited ways to make ATP from glucose. How do they make most of their ATP from glucose?

They get the majority of their ATP from glucose by glycolysis.

25 (3pts) Patients with severe PDHD have elevated blood lactic acid. State why this is the case (one sentence). Write the reaction that forms lactic acid. Name the enzyme and write the reaction, including structures of the three-carbon reactants and products.

a) your sentence: Lack of PDH activity causes a buildup of unreacted pyruvate, which is then converted into lactic acid by lactate dehydrogenase

b) your reaction:

\[
\text{Pyruvate} + \text{NADH} + \text{H}^+ \rightarrow \text{Lactate} + \text{NAD}^+ 
\]

\[\Delta G^{\circ} = -25.1 \text{ kJ/mol}\]
Thinking about drinking (6 points)
Ingested ethanol is detoxified by the liver to harmless acetate. This occurs by a two reaction pathway, in which ethanol is converted into acetaldehyde, and then acetaldehyde is converted into acetate.

27; 2 pts) First, draw the structures of the three molecules.

\[
\text{ethanol} \quad \text{acetaldehyde} \quad \text{acetate}
\]

The first step (ethanol to acetaldehyde) is catalyzed by alcohol dehydrogenase. The second step (acetaldehyde to acetate) is catalyzed by aldehyde dehydrogenase. Both use NAD+ as a substrate. Write the balanced reaction for the first step showing the two structures; and include NAD+ in the reactions.

28; 2 pts) Alcohol dehydrogenase reaction:

\[
\text{alcohol dehydrogenase reaction:}
\]

Antabuse®, is used clinically as a deterrent to drinking. It is an inhibitor of aldehyde dehydrogenase, and patients on Antabuse get very ill if they drink alcohol.

29) When a person on Antabuse drinks ethanol, what molecule do you expect to accumulate? __ACETALDEHYDE (yuk!)__

30; 2 pts) The \( E^\circ \) for the acetaldehyde/ethanol half reaction is \(-0.197 \text{ eV} \). Write the balanced half reaction, including electrons.

\[
2e^- + \text{acetaldehyde} + 2H^+ \rightarrow \text{ethanol}
\]

31) From the \( E^\circ \) value, say which direction the half-reaction runs spontaneously, justifying your answer (one sentence).

The half reaction is spontaneous in the direction of ethanol oxidation, since the \( E^\circ \) for the reduction is negative, meaning it has a positive \( \Delta G^\circ \).
31 (1.5pts) The Krebs cycle results in the production of 3 distinct energy-rich molecules. What are they?

NADH  FADH$_2$  GTP

32 (3pts) CO$_2$ is produced by the Krebs cycle, along with more useful things. Write the first reaction of the Krebs cycle that produces CO$_2$. Include the name of the enzyme, the names of all the reactants and products, and the structures of the pathway molecules involved.

33 (1.5pts) A later Krebs cycle reaction produces the molecule succinate. Show the reaction that produces succinate, including the enzyme name, and the names of all the reactants and products. No need for structures in this reaction.

Succinyl-CoA + GDP + Pi $\rightarrow$ Succinate + GTP + CoASH

34) Suppose you are studying the Krebs cycle in isolated cells using labeled molecules that can be detected. If 50 µmoles of acetyl groups are consumed by the Krebs cycle in one of your experiments, how much CO$_2$ is produced in that experiment?  

100 µmoles of CO$_2$

35 (2pts) What are the two enzymes of the Krebs cycle that produce CO$_2$ in the course of the reactions they catalyze?

First enzyme: _isocitrate dehydrogenase_

Second enzyme: _α-ketoglutarate dehydrogenase_

36 (3pts) List the three enzymes in the Krebs cycle that undergo allosteric regulation?

1) _________ citrate synthase _____________

2) _______α-ketoglutarate dehydrogenase __________

3) _____________isocitrate dehydrogenase ___________

37) How many reactions of the Krebs cycle use oxygen as a substrate? ___0!! (none!)___
The glyoxylate cycle in pathogen virulence (10 points)

In 2001 Lorenz and Fink (Nature (2001) 412 83-85) demonstrated that a pathogenic fungus needs the glyoxylate cycle to efficiently kill its mammalian hosts. Furthermore, the tuberculosis bacterium also requires the glyoxylate cycle to be fully infectious (McKinney et al., Nature (2000) 406, 735-738). These are very important observations, since processes that are needed by pathogens but absent in us are potential targets for clinical intervention. So you can see that the things we talk about in class can have medical relevance.

38) What is the glyoxylate cycle? Write one sentence about its function in the organisms that are lucky enough to have it.

The glyoxylate cycle is used to bring carbons from acetyl groups (in AcCoA) into anabolism; for example with this pathway fat-derived acetyl CoA can be converted into glucose

The glyoxylate cycle has two unique enzymes that are distinct from Krebs cycle enzymes. They are called isocitrate lyase, and malate synthase.

39 (3pts)) Write the reaction catalyzed by isocitrate lyase, including the structures of the pathway molecules.

40) From what you know about the regulation of the glyoxylate and Krebs cycles, what would you expect the activity of isocitrate dehydrogenase to be if the activity of isocitrate lysase in the same cell is high? One sentence, please.

When the glyoxylate cycle is high, the Krebs cycle is kept low since the same regulator control these two enzymes in opposite ways.

41 (3pts)) Write the reaction catalyzed by malate synthase. Include structures of the pathway molecules.

42) For each turn of the glyoxylate cycle, how many acetyl groups enter? _______2_____

43) For each turn of the glyoxylate cycle, how many CO₂ are produced? __0; none__
Gotta have glycolysis... (14 points)

44 (4pts) During glycolysis, two different phosphorylated molecules are produced that are capable of transferring phosphate to ADP to make ATP. Draw the structures of these two molecules below, in the order they are produced in the pathway.

\[
\begin{align*}
1,3\text{-Bisphosphoglycerate} & \\
\text{FIRST} & \\
\end{align*}
\]

\[
\begin{align*}
\text{Phosphoenolpyruvate} & \\
\text{SECOND} & \\
\end{align*}
\]

45 (2pts) Write the names of the enzymes responsible for the production of each of these high energy phosphorylated molecules.

- \text{glyceraldehyde-3-phosphate dehydrogenase} (FIRST ENZYME)
- \text{enolase} (SECOND ENZYME)

46 (6pts) When glucose is first broken into two molecules, the result is two different three carbon molecules. Draw their structures below and say the full names.

- \text{Dihydroxyacetone phosphate}
- \text{Glyceraldehyde 3-phosphate}

47) Each of these molecules has a phosphate, yet glucose, from which their carbons came, has no phosphate. What molecule(s) do the phosphates on each molecule come from?

The phosphates both come from \text{ATP}, used in the priming reactions.

48) In microorganisms, ethanol is produced when cells are primarily using glycolysis for energy. Similarly, lactic acid is produced in animal tissues that are mainly using glycolysis for energy. In both cases, the production of these molecules must occur for glycolysis to proceed. Why? (One sentence)

\textbf{Ethanol or lactate production each convert NADH to NAD\textsuperscript{+}, which is needed for continued glycolysis.}
That other glucose pathway…. (7 points)

The pentose phosphate pathway is another fate for glucose that is distinct from The G Word, but still very important.

49 (2pts) The pathway has a first phase of 5 reactions, that involves oxidation. What are the two main, useful products of this part of the pentose phosphate pathway?

__________NADPH__________ ribose-5-phosphate (or ribose)________

50 (3pts) The first reaction of the pentose phosphate pathway involves a very important enzyme called glucose-6-phosphate dehydrogenase. Write the first reaction, including the structures of the pathway molecules. Hint: a lactone is a cyclical ester.

51 (2pts) Which two large biomolecules, central to life, are made from one of the products of the pentose phosphate pathway?

_______RNA________ and _______DNA__________
One small step for a mitochondrion, one giant leap for mitochondria… (9 points)

Here are 4 cellular locations, including the three compartments of the mitochondrion:

A  mitochondrial outer membrane
B  mitochondrial inner membrane
C  mitochondrial matrix
D  the cytosol

(5 pts) For each phrase below, write the one letter that is best associated with the phrase:

52) _____ A _____ permeable to small ions and molecules
53) _____ D _____ location of glycolytic enzymes
54) _____ D _____ where lactic acid is formed.
55) _____ C _____ location of Krebs cycle enzymes
56) _____ D (best answer) _____ location of glyoxylate cycle, if present
57) _____ B _____ location of cytochromes
58) _____ B _____ where ubiquinone is found
59) _____ A _____ contains porins
60) _____ B _____ highly impermeable to small molecules and ions
61) _____ C _____ where the PDH complex is found

62 (4 pts) Sketch a cross section of the mitochondrion below, labeling the key parts. 
This is a bit fancier than I usually draw but easier to put on the electronic key than the capsule with a squiggle that I have drawn in class many times.