ENZYMES and KINETICS (10 points this page)

A substrate S is converted into product P. When an enzyme is added to the reaction, the activation energy is lowered. Use single sentence answers for the following:

1) What is the effect of addition of enzyme on the forward reaction (S to P) and the reverse reaction (P to S)?

2) What is the effect of adding the enzyme on the equilibrium constant for S conversion into P?

3) Suppose that the enzyme lowers the activation energy by 5kJ per mole. What letter best describes the change in rate of the S to P reaction compared to when no enzyme is present?

    your choice: __________

    A) it is faster by a factor of $e^{(5,000/RT)}$  
    B) it is faster by a factor of $(5,000/RT)$
    C) it is slower by a factor of $e^{(5,000/RT)}$  
    D) the change depends on the original $E_{act}$

Two versions (isozymes) of the same enzyme convert substrate S into product P. They each have the same $k_{cat}$, but isozyme M (in muscle) has a $K_m$ of 5 uM and isozyme L (in liver) has a $K_m$ of 20 uM. Answer the following questions about this situation.

4) At what substrate concentration will M be half-saturated?

5) What fraction (between 0 and 1) of saturation will enzyme M have at 20 uM substrate?

6) What fraction of saturation will enzyme L have at 20 uM substrate?

7) What is the ratio of maximal rates, $L_{max}/M_{max}$, for the two enzymes? (Think first…)

8) (3 points) Write the general reaction for phosphorylation of an enzyme E. Use –OH to represent the R group that actually receives the phosphate. Make sure to include all necessary substrates and products, and include the general name of the type of enzyme that catalyzes this reaction.
ENZYMES and KINETICS cont’ (10 points this page)

The graph is a picture of a Lineweaver-Burk plot, with data from two different enzymes, I and II, plotted.

9) (2 points) In this sort of plot, each axis is a function of either substrate concentration or rate. Indicate what each axis represent in the space below

The X (horizontal) axis is:

The Y (vertical) axis is:

10) Which of the two enzymes, I and/or II, follows Michaelis-Menton kinetics? ____________

11) (2 points) Why do you say the answer in 10)? (One sentence please)

12) Where on this plot does one find the saturated rate for an enzyme? Write below please.

Allosteric enzymes are often encountered in the study of metabolic regulation. Below is a simple rate plot, in rate plotted against substrate concentration S.

Sketch in three curves, making sure to indicate which is which with labels.

13) Draw a curve for a typical allosteric enzyme, label it E

14) Draw a new curve for E when an allosteric activator is added, and label it A

15) Draw a third curve for E when an allosteric inhibitor is added and label it I

16) What protein structural feature is nearly always associated with kinetic behavior of this sort?
OXIDATION REDUCTION  (10 points this page)

17 (4 points) Glyoxylate can be oxidized to the two carbon, dicarboxylic acid oxalate. This is one source of the material in kidney stones. Starting with the structure of glyoxylate below, write a reaction for the oxidation of glyoxyate by NAD. Include any water or H\(^+\) you need to balance the reaction

![Glyoxylate Structure]

(Hey! These are the reactions from last year’s midterm! ) The following four half-reactions have the standard reduction potentials listed.

A) \(\text{KG} + \text{CO}_2 + 2\text{H}^+ + 2e \rightarrow \text{isocitrate} \quad E^{\circ} = -0.38\)

B) \(\text{NO}_3^- + 2\text{H}^+ + 2e \rightarrow \text{NO}_2^- + \text{H}_2\text{O} \quad E^{\circ} = 0.421\)

C) \(\text{crotonyl-CoA} + 2\text{H}^+ + 2e \rightarrow \text{butryl-CoA} \quad E^{\circ} = -0.015\)

D) \(\text{Pyruvate} + 2\text{H}^+ + 2e \rightarrow \text{lactate} \quad E^{\circ} = -0.185\)

There are 4 acceptors and 4 donors in this collection of reactions. Answer the following questions

18) Which molecule in this collection of 8 (donors and acceptors) is the best oxidizing agent?

19) Which molecule in this collection of 8 is the best reducing agent?

20) List the letters for the reaction or reactions that would spontaneously reduce H\(^+\) ion at standard conditions

21) (3 points) For reaction D, show the relationship between \(\Delta E^{\circ}\) and the \textit{equilibrium concentrations} for this half reaction. Use letters for any constants (eg A for avogadro's number)
An inborn error of glycolysis (10 points this page)

One of the most powerful tools in the study of metabolism is use of radioactive atoms. The next questions will use a labeled molecule to understand glycolysis in the cells of normal individuals, and those with an inherited disease.

22) (2 points) 1-$[^{14}C]$-fructose-6P is added to a cellular lysate with a fully functional glycolytic pathway. Draw a molecule of labeled pyruvate that would result from the labeled molecule ending up in that product. Just the pyruvate please…

23) (2 points) Does every molecule of pyruvate derived from a labeled fructose have a labeled carbon? Yes or no, and explain your answer

There is a severe inherited disease caused by a null allele of the triose phosphate isomerase (TPI) gene. You know, the 5th enzyme of the glycolytic pathway. Homozygotes produce NO TPI enzyme activity.

24) (4 points) Write the reaction that TPI catalyzes, including structures of reactant(s) and product(s)

25) (2 points) 1-$[^{14}C]$-fructose-6P is added to a cell lysate completely deficient in TPI, and glycolysis is allowed to proceed. Is there still label found in pyruvate? Why or why not?
An inborn error of glycolysis (cont’) (10 points this page)

26) (3 points) 1-[14C]-fructose-6P is added to a cell lysate deficient in TPI, what would you predict the main molecule with labeled 14C to be? Justify your answers with a picture and/or a sentence.

27) How many ATP are consumed for each glucose that goes down this mutant pathway? __________

28) How many ATP are produced from each glucose that goes down this mutant pathway __________

29) How many reduced carrier molecules are made per glucose by the TPI-deficient pathway? __________

30) What is this reduced carrier molecule called ______________

31) How many molecules of pyruvate are formed per glucose by the TPI-deficient pathway __________

32) (2 points) Explain the number you gave in 31) below with one sentence:
Glucose coming and going (10 points this page)

Nature’s Sugar Bowl Glycogen is a key glucose storage molecule in mammals.

33) (4 points) Using the picture below of a single non-reducing end of glycogen, write the glycogen phosphorylase reaction, showing any substrates and products, but don’t worry about drawing the remaining glycogen molecule.

34) (2 points) How is the molecule produced by glycogen phosphorylase then put into the glycolytic pathway? Write the relevant reaction, no structure required. (Hint: a mutase is required)

35) (2 points) Glucose regulates glycogen phosphorylase allosterically, by making glycogen phosphorylase more susceptible to the action of another regulatory enzyme. What kind of enzyme is this?

36) (2 points) Write the general reaction catalyzed by the enzyme involved in glucose regulation of glycogen phosphorylase. You can use “P” to indicate the protein substrate.
Glucose coming and going (cont’’) (8 points this page)

One of the fates of glucose distinct from glycolysis is for the production of ribose.

37) (2 points) What is the name of this pathway?

38) (4 points) Write the first reaction including the names of products, reactants and cofactors

39) (2 points) The second part of this pathway, after ribose production, involves a remarkable collection of interconversions of 4, 5, 7 and 3 carbon sugars. Two types of enzymes are involved in all of those fancy conversions.

The enzyme that transfers 2 carbon units is called: ________________________

The enzyme that transfers 3 carbon units is called: ________________________

(this is just a little dead space….)

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Krebsian philosophy (10 points this page)

One of ways that Krebs cycle molecules are replenished is by a small group of so-called anaplerotic reactions. One of these mentioned in class that is very important to mammals is catalyzed by phosphoenolpyruvate carboxykinase, called PEP-CK for good reason.

40) (2 points) Write the PEP-CK reaction, including all substrates and products. No structures needed.

41) We have said numerous times in class that “you can’t use the Krebs cycles for net conversion of acetate into glucose” Why is this the case? (One sentence)

42) Most of the energy-rich molecules derived from the Krebs cycles are involved in carrying electrons. However, an energy-rich molecule that is not ATP is directly produced by “substrate-level phosphorylation”. What is this molecule?

43) (2 points) Write the Krebs cycle reaction that is responsible for this substrate level phosphorylation. Include the name of the synthetase (he hinted) that catalyzes it. Names are fine… no need for structures, beautiful as the may be.

44) When an organism does want to use acetate for net synthesis of sugars, an alternate cycle is used. In class we referred to this process as an “end run around the Krebs cycle” What is the name of this metabolic process?

45) (2 points) What is the first reaction of this alternate route for acetate carbons? Write the reaction and the name of the enzyme that catalyzes it. Just names, but include all substrates and products.

46) Why does this alternate pathway allow net synthesis of things like glucose from acetate?
Cofactor coop (10 points this page)

Fill in the blanks Write the cofactor or carrier that best goes with the brief description:

47) Metal ion needed for practically every enzyme that uses ATP or ADP ________________

48) Carrier for sugar molecules, used in the conversion of galactose into glucose ________________

49) Undergoes disulfide oxidation/reduction cycle in order to convert pyruvate into acetyl-CoA. It acts as a “rocker arm” in the enzyme we studied ________________

50) Used in the carboxylation of many molecules by allowing activation of CO\(_2\) ________________

51) (2 pts) Reduced form of the carrier that picks up most of the electrons from Krebs cycle ______

Molecules at and Exhibition For the structures below, select the letter that corresponds best to the structure.

A) NAD  B) TPP  C) CoA  D) biotin  E) FAD  F) ADP  G) PEP  H) none

52) ________  53) ________

54) ________  55) ________
Veracity or Fallaciousness (T or F) (12 points this page)

56) ______ The inner membrane of the mitochondrion is freely permeable to ions

57) ______ Competitive enzyme inhibitors do not affect the Vmax of a reaction

58) ______ Krebs cycle occurs in the cytoplasm of mammalian cells.

59) ______ A positive $\Delta E^\circ$ means a non-spontaneous reaction

60) ______ An increase in activation energy will change an equilibrium

61) ______ Regulated metabolic reactions are often far from equilibrium.

62) ______ All spontaneous reactions give off heat, that is, have a negative delta H

63) ______ The Krebs cycle only functions in catabolism

64) ______ ATP is oxidized to ADP and phosphate by water

65) ______ There are mammalian cells that are almost totally dependent on glycosis for their energy

66) ______ Muscles convert pyruvate into ethanol to regenerate NAD$^+$

67) ______ Nelly is a big fan of glycogen