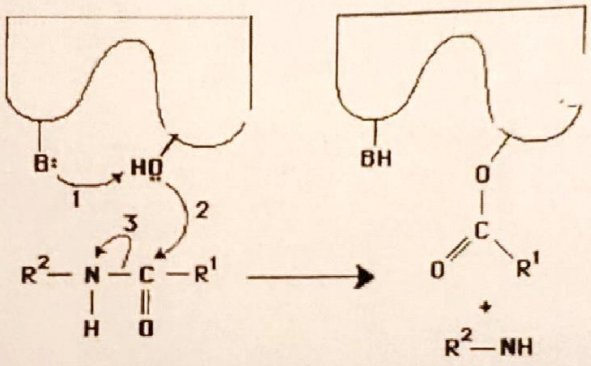
CH/BI 308 Biochemistry Exam II Fall 2020 Name: Key

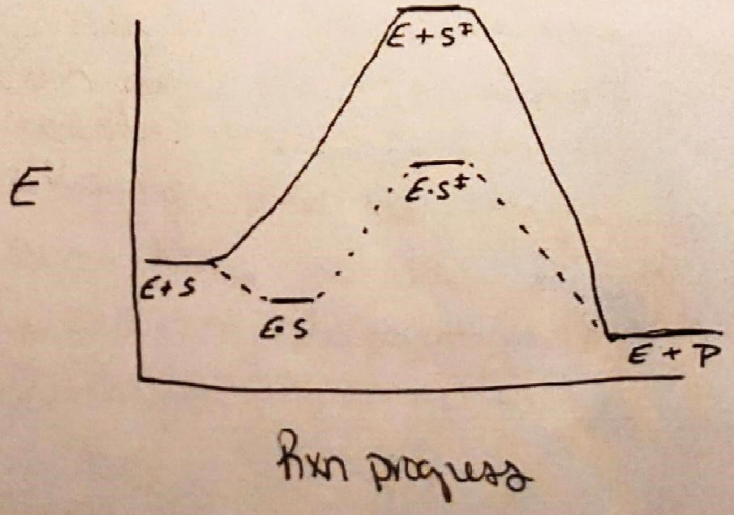
Please read the directions carefully. No cell phones or other electronic devices except for calculators are allowed. The use of our course textbook is allowed; however, the use of notes, workbook activities, the internet or peer collaboration is prohibited during the exam and the use of those materials would be a violation of the BSC Honor Code. Students suspected of violating the honor code will be reported to the honor council for review. This exam will be posted on moodle at 8:00 AM on the scheduled day of the exam. Completed exams must be turned in via the TurnItIn link on moodle by 11:59 PM Central on the scheduled day of the exam. No late exams will be accepted. If you have a question, I will be available via Teams during our normal scheduled class time or can be reached via email at khayden@bsc.edu. Good luck, take your time, and read carefully!

25 Honor Code:_____

1. The reaction below shows a general method used by proteases to catalyze the cleavage of a peptide bond. In this figure, 1 and 2 are generic proton donors and acceptors that are part of the enzyme active site. The rest of the polypeptide chain, which surrounds the substrate in the active site and sequesters it from bulk solvent, is not shown for clarity. (20)



a. Draw a reaction coordinate diagram (energy vs reaction progress) that shows the enzyme-catalyzed reaction (as shown above) and uncatalyzed reaction. Label the relative energy of the reactants, transition state and products (both bound and unbound to the enzyme) for each diagram. (5)



b. Using the diagram you drew above, explain how enzymes catalyze (or speed up) reactions.

(5) Enzymes bind and stabilize the transition state ($S^{\ddagger}$) lowering the energy of activation allowing the reaction to happen faster.

c. What is (are) the mechanism(s) of catalysis utilized by the enzyme depicted in the figure above?

(5) ① general acid/base
② Covalent

10 (5)

d. Propose specific amino acids that could best serve the functions of 1 and 2 in the enzyme.

① A.A. that have side chains that can act as a general base or acid, His would be the best b/c pKa is close to physiological pH but Asp. or Glu might work as well to deprotonate (2)
② anything w/ OH → Ser, Tyr (maybe Cys w/ SH).

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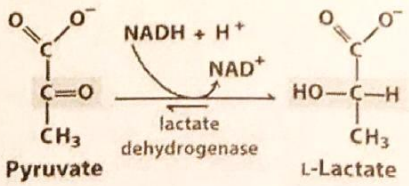
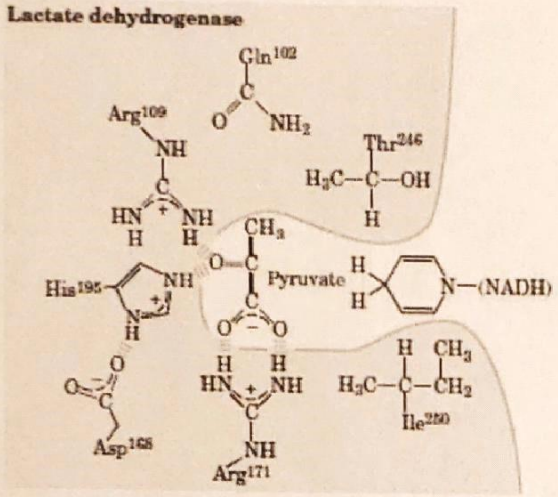
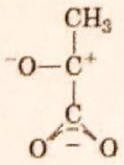
2. Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate with NADH to form lactate. The overall reaction (right), a schematic of the enzyme's active site (below, left), and the structure of the strongly polarized transition state of the reaction (below, right) are shown.







transition state of pyruvate in the reaction

Muireanne created a mutant form of LDH in which Arg109 is replaced with Gln. This mutant shows only 25% of the pyruvate binding and 0.07% of the activity of wild-type enzyme.

a. Why can pyruvate binding still occur at a significant level despite the mutation?

The R group of Gln still has functional groups that can for H-bonds w/ Pyruvate O.

b. In the Gln mutant, what is the biochemical explanation for why the activity is so low despite pyruvate still being able to bind at a significant level?

Gln does not have ~~formal~~ charge like Arg, plus its R-group is shorter so orientation w/ other binding groups is off ∴ not as stabilizing

c. Angelina created a mutant form of LDH in which Ile250 is replaced with Gln. This mutant shows significantly reduced binding of NADH. Provide a plausible biochemical explanation for this result.

Ile has hydrophobic CH_3 that interacts w/ hydrophobic ring of NADH. Replacing w/ polar R group of Gln would have a repulsive effect.

(10) 3.

Enzyme	Substrate	K_M (M)	k_{cat} (s^{-1})	k_{cat}/K_M ($M^{-1} \cdot s^{-1}$)
Acetylcholinesterase	Acetylcholine	9.5×10^{-5}	1.4×10^4	1.5×10^8
Carbonic anhydrase	CO_2	1.2×10^{-2}	1.0×10^6	8.3×10^7
	HCO_3^-	2.6×10^{-2}	4.0×10^5	1.5×10^7
Catalase	H_2O_2	2.5×10^{-2}	1.0×10^7	4.0×10^8 ←
Chymotrypsin	*N*-Acetylglycine ethyl ester	4.4×10^{-1}	5.1×10^{-2}	1.2×10^{-1}
	N-Acetylvaline ethyl ester	8.8×10^{-2}	1.7×10^{-1}	1.9
	N-Acetyltyrosine ethyl ester	6.6×10^{-4}	1.9×10^2	2.9×10^5
Fumarase	Fumarate	5.0×10^{-6}	8.0×10^2	1.6×10^8
	Malate	2.5×10^{-5}	9.0×10^2	3.6×10^7
Urease	Urea	2.5×10^{-2}	1.0×10^4	4.0×10^5

(5) a. From the table above, which enzyme/substrate pair could be classified as the "most efficient?" Include one sentence that justifies your answer.

Catalase w/ H_2O_2 b/c it has the largest k_{cat}/K_M (or the highest catalytic efficiency)

(5) b. With the substrate, urea, urease has a k_{cat} of 1.0×10^4 s^{-1}. Write a one sentence definition of what that actually means.

k_{cat} is the turn over number, or the number of reactions an enzyme can catalyze in a given unit of time

(15/15)

4. What are the two reactions in glycolysis in which aldose to ketose isomerization is catalyzed by an enzyme?

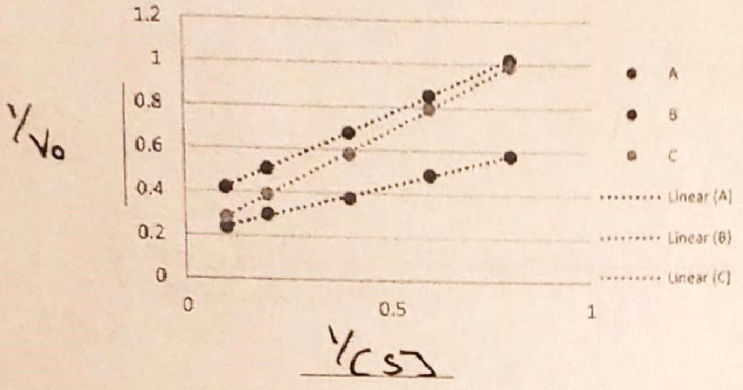
(5) G6P ⇌ F6P by PGI $\Delta G^\circ = +2.2$ GAP ⇌ DHAP by TPI (or Tim) $\Delta G^\circ = +7.9$

a. For both reactions the ΔG° is positive. Briefly explain how the reactions are able to proceed without the input of additional energy.

(10/10) By keeping the [product] much much lower than [Reactant] so eq shifts to be product favored and ΔG becomes negative. This is done by rapidly using the product up in the next step of glycolysis

30
(28)

5. Dr. Burke is trying to develop a drug that targets and inhibits the enzyme, HIV protease. One of his undergraduates synthesized two new compounds that seem promising. The kinetic data below were obtained for HIV Protease in the absence of inhibitor (trial A), and in the presence of the two new inhibitors (B and C) at inhibitor concentrations of 5mM.



The data for each trial is fitted as follows:

A: y=0.4843x + 0.1951

B: y=1.0062x + 0.1861

C: y=0.8582x + 0.3369

(5) a. Properly label the axis of the graph above.

See above

(10) b. Determine V_{max} and K_M for the enzyme for each trial. (Show your calculations for full credit)

	Vmax	Km
A	5.126 mm/s	2.482 mM
B	5.373	5.407
C	2.968	2.547

(5) c. Determine the type of inhibition for trial B and C, and provide a reason why you chose what you did.

B is competitive b/c Vmax is constant, $K_{M_{app}}$ ↑

C is mixed (or noncompetative) b/c $V_{max_{app}}$ ↓'s $K_{M_{app}}$ is constant.

10
(8) d. Because Dr. Burke is targeting HIV Protease for his new treatment pathway, make a prediction as to which inhibitor, B or C, makes a better candidate for treatment? Explain your reasoning.

C can not be overcome by ↑ [S] so for something like a virus this would probably be ideal.

20
(19)

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6. The hydrolysis of ATP is thermodynamically favorable, with a release of ~32kJ/mol of energy.

$ATP^{4-} + H_2O \rightarrow ADP^{3-} + HPO_4^{2-} + H^+ \quad \Delta G = -32kJ/mol$

a. Give three reasons to explain why this reaction is exergonic.

(5)

① Products are more resonately stabilized
② entropically favored
③ electrostatic strain is ↓ in products.

b. If this reaction is thermodynamically favorable, explain why the spontaneous hydrolysis of ATP rarely occurs in the cell.

(5)

kinetically it is very slow

10
(8)

c. Specifically explain how the kinases lower the energy of activation for this reaction?

Mg^{2+} is used to help shield the negative charges on ATP while the terminal phosphate is cleaved & transferred to substrate. Mg^{2+} shielding allows H_2O to nucleophilically attack the phosphate which results in phospho-anhydride bond.

15
(10)

7. Why is fructose metabolized metabolized more quickly than glucose? What potential health problem does this promote? Explain.

Fructose metabolism bypasses PFK-1 in the liver and can activate pyruvate kinase by largely ↑[PEP] & [F1,6BP] and is unregulated since it bypasses two major step pants in glycolysis. The excess pyruvate made is then often stored as fat and can cause fatty liver disease.

(10)

8. Sara Beth wanted to see what would happen to glucose that is isotopically labeled at carbon 2 (5-^{14}C-glucose) when given to metabolically active liver cells in a cell culture.

(10) a. If the cells where undergoing cellular division, predict where Sara may find the isotopic labeled carbon and explain why. (Consider all possibilities).

- In nucleic acids (if PPP is going)
- As the carbonyl carbon of pyruvate (via glycolysis)
- as C2 or C5 of glucose (via gluconeogenesis)

(10) b. If the cells where in a resting state, predict where Sara may find the isotopic labeled carbon and explain why. (Consider all possibilities).

- glycogen (@ C2) for storage (if resting ? ATP needs are ~~or~~ met)
- pyruvate @ carbonyl carbon via glycolysis if ATP is needed or if glycogen stores are maxed, then it will continue to fat storage.

(10) 9. If you incubate ^{14}C-CO_2 with liver extracts capable of performing gluconeogenesis, where does the radioactive label end up? Explain.

While CO_2 is used in the first steps of gluconeogenesis as ~~PEP~~ pyruvate is converted back to PEP, it is released again @ the end & the isotopic carbon would only show up in CO_2.

10. Give an example of an isozyme studied in glycolysis and explain, relative to your example, how the presence of isoforms in different tissues allows a unique avenue of metabolic control. Sketch the [S] versus v_o curves for each isozyme to help illustrate your answer.

a variety of answers

(15)

11. Inorganic fluoride inhibits enolase.

(5) a. In an anaerobic system that is metabolizing glucose as a substrate, which of the metabolites in glycolysis would you expect to increase in concentration immediately following the addition of fluoride? Explain.

2PG, 3PG, 1,3BPG, DHAP, GAP & F16BP b/c all steps between enolase & aldolase are reversible

(5) b. Which of the metabolites in glycolysis would you see increase in concentration 10 minutes after addition of fluoride? Explain.

F6P & G6P. The build up of F16BP will eventually inhibit PFK1 so metabolites between PFK1 & hexokinase will build up

(5) c. Hours after the addition of fluoride what would you expect to occur with the levels of glucose in the cell? Explain.

[Glucose] ↓ in the cell as G6P will inhibit hexokinase & glucose will either be stored as glycogen or go elsewhere in the body (back into blood)