

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise and clear. I have given you more space than you should need. There is extra space on the last page if you need it.

You will need a calculator for this exam. No other study aids or materials are permitted.

Generous partial credit will be given, *i.e.*, if you don't know, guess.

Useful Equations:

$$v_0 = V_{\max}[S]/(K_M + [S])$$

$$pH = -\log([H^+])$$

$$\Delta G^\circ = -RT \ln K_{eq}$$

$$K_a = [H^+][A^-]/[HA]$$

$$\Delta G = \Delta H - T\Delta S$$

$$pH = pK_a + \log([A^-]/[HA])$$

Honor Pledge: At the end of the examination time, please write out the following sentence and sign it, or talk to me about it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1. (30 pts) Hemoglobin and oxygen transport.

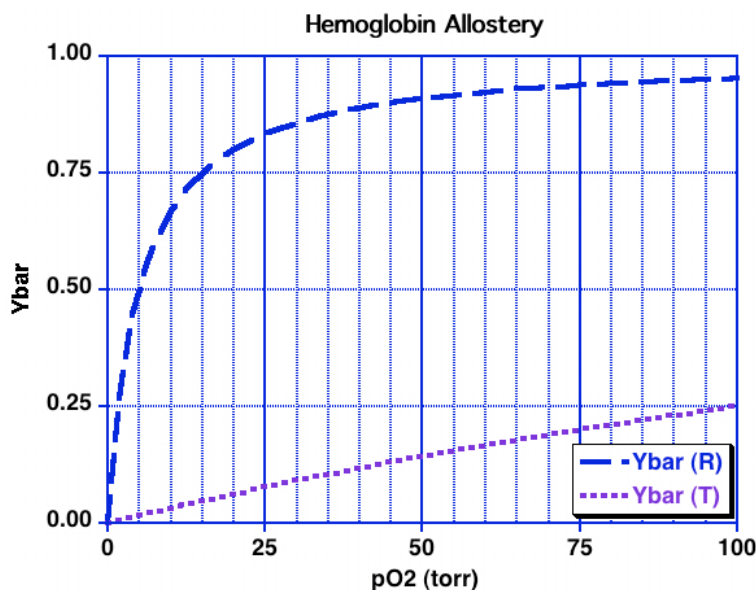
(a; 3 pts) Why have myoglobin and hemoglobin evolved to bind gaseous ligands in a bent conformation relative to the gas-iron bond?

To prevent gaseous ligands such as O₂ from binding too tightly (irreversibly, as does carbon monoxide).

(b; 6 pts) The graph at the right shows hypothetical "pure R" and "pure T" state binding curves. Sketch in the binding curve for Hb at pH 7.6, with a p50 of 26 torr. Sketch in the binding curve at pH 7.2. You do not need to know the p50: I am just asking for which direction the curve will shift.

Hb at pH 7.6: Sigmoidal curve (2 pts), reaches 0.50 at pO₂ = 26 torr (2 pts).

Hb at pH 7.4: sigmoidal curve, shifted to the right/down (lower affinity) compared to pH 7.6 (2 pts)



Score for the page _____

(c; 16 pts) Blood picks up oxygen in the lungs. What happens to each of the following molecules during this process? Include in your answers how each species affects the R $\leftrightarrow$ T interconversion of hemoglobin.

Oxygen:

Binds to T state of Hb, converts it to R state (2 pts), leading to cooperative binding to end up with nearly complete binding of oxygen (2 pts)

Carbon dioxide/bicarbonate:

Bicarbonate flows into red blood cells (RBCs) and is converted to H₂O + CO₂ by carbonic anhydrase, with the process being driven by equilibration with air at low pCO₂ (2 pts). CO₂, which forms a carbamate when bound to Hb, is released from Hb in the lungs, leading to Hb conversion from T $\rightarrow$ R (carbamate stabilizes T) (2 pts).

Protons:

Combine with bicarbonate to form CO₂: exhalation of CO₂ effectively neutralizes the blood (2 pts).

Protonation stabilizes the T state of Hb, so deprotonation of Hb accompanies oxygenation. (2 pts)

Chloride ion:

Flows out of RBC as bicarbonate flows in, to balance charge (the “Band III protein” is an antiporter that exchanges the two). (2 pts)

Chloride stabilizes the T state, so loss of Cl⁻ stabilizes the R state, encouraging O₂ binding. (2 pts)

(d; 5 pts) The Bohr Effect refers to the observation that Hb is a stronger acid when bound to oxygen than when it is not. Why does the Bohr effect make sense in terms of the physiology of oxygen transport?

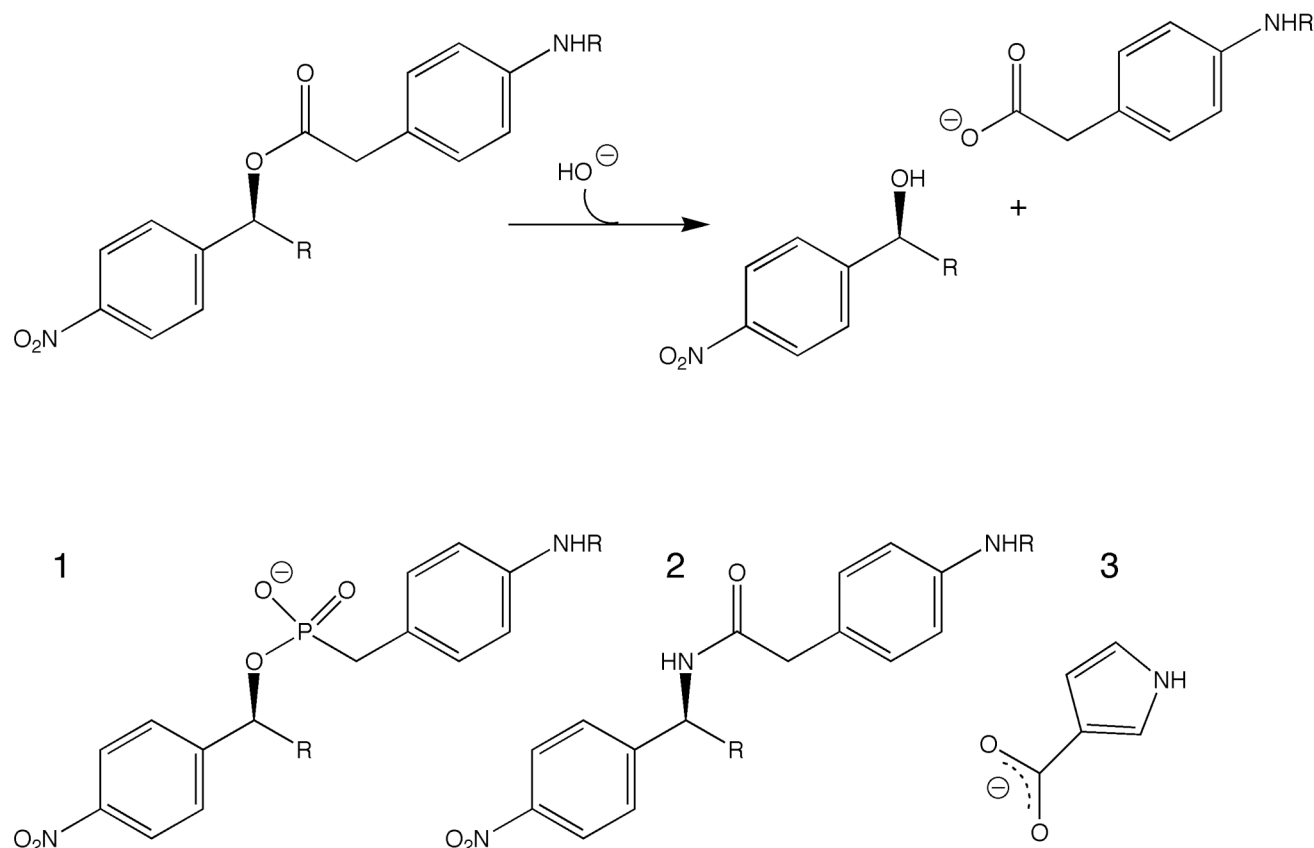
The production of CO₂ in muscle acidifies the tissues and the blood going through. (2 pts)

Since oxygen binding is coupled to release of protons, uptake of protons is coupled to oxygen release. (1 pt)

Therefore O₂ is released most efficiently in muscles that are working hard. (2 pts)

2. (15 pts) Enzyme kinetics

(a; 8 pts) Why are transition state analogs generally better (tighter-binding) enzyme inhibitors than substrate analogs? Which one of the inhibitors shown at the bottom below looks like it might be a transition state analog for the hydrolysis reaction at the top, and why?



Enzymes have evolved to stabilize transition states more than they do substrates: otherwise they would not decrease the free energy of activation (2 pts). Therefore, the transition state has better structural complementarity to the active site than the substrate, and a transition state analog binds better than a substrate analog, therefore is a more effective competitive inhibitor (2 pts).

Compound 1 (2 pts) resembles the charged, tetravalent intermediate in an ester hydrolysis reaction (2 more points). Compound 2 is a substrate analog, compound 3 is unrelated.

(b; 7 pts) Write down the Michaelis-Menten equation. We will not derive it here, but you should remember the ideas that made the derivation possible. To which critical intermediate did we apply the Steady State Approximation? What is the Steady State Approximation?

+2 From the first page: $v_0 = V_{\max}[S]/(K_M + [S])$

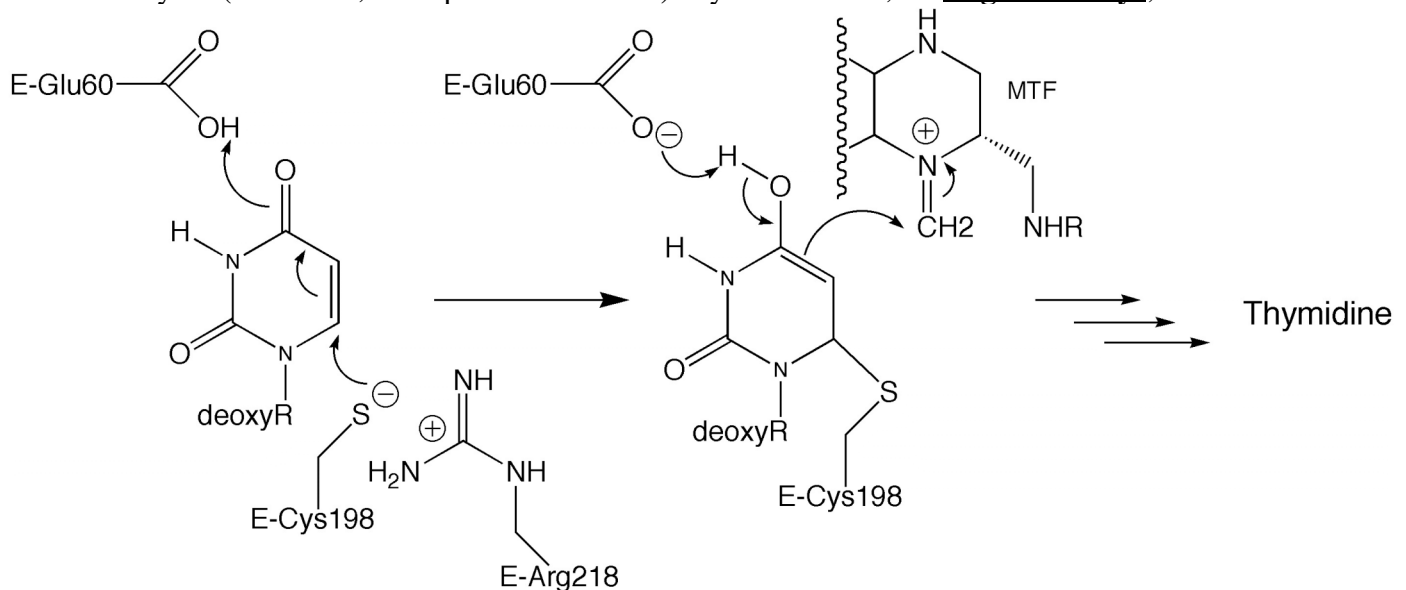
+2 The ES complex is the intermediate: the SSA applies to intermediates that have rapid decay paths available.

+2 The SSA says that the concentration of the intermediate is constant during most of the reaction.

+1 It is useful because we can set the net rate of change = 0 and therefore solve for the concentration of the intermediate.

3. (25 pts) Enzymatic catalysis and inhibition

(a; 11 pts) A simplified version of the early steps of the reaction catalyzed by thymidylate synthase (TS) is shown below. Three of the active site residues are Cys 198, Arg 218, and Glu 60. Which of the common modes of enzymatic catalysis that we discussed in class is exemplified by each residue (one answer each)? Which one of the following mutations do you think is most likely to give an active enzyme (circle one, no explanation needed): Cys 198 → Val, **+2 Arg 218 → Lys**, Glu 60 → Thr?



+3 Cys 198 is responsible for covalent catalysis - note S⁻ acts as a nucleophile

+3 Arg 218 does electrostatic catalysis, stabilizing the nucleophilic S⁻

+3 Glu 60 does acid-base catalysis, doing proton transfers to and from the uridine to stabilize enolic intermediates

(b; 7 pts) The scheme below (page 722 of Berg 6e) shows inhibition of TS by FdUMP, which is an irreversible inhibitor used in chemotherapy. What is the difference between reversible and irreversible inhibition? How can one differentiate experimentally between a reversible inhibitor and an irreversible inhibitor?

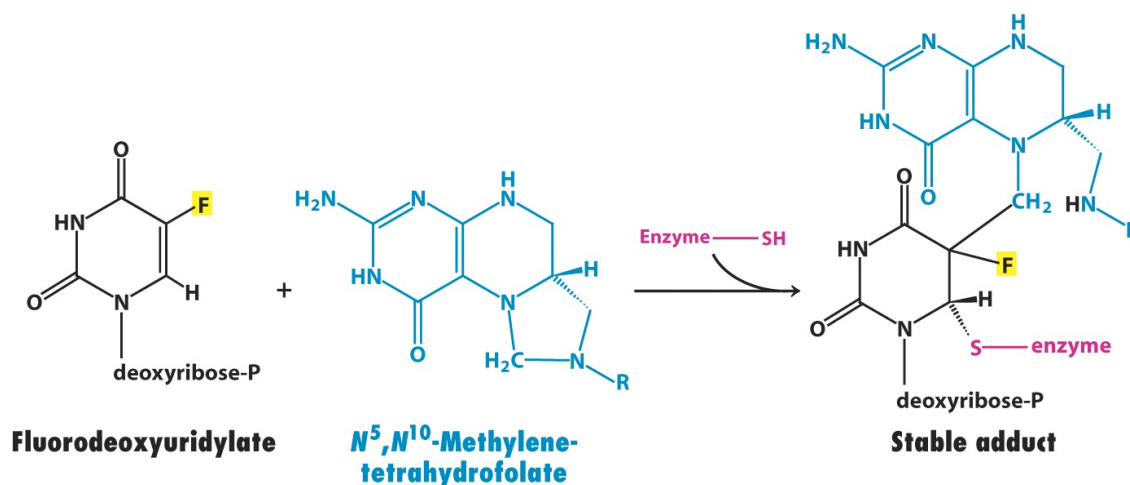


Figure 25-14
 Biochemistry, Sixth Edition
 © 2007 W. H. Freeman and Company

- +3 This is an irreversible inhibitor: the drug makes a permanent covalent adduct with the enzyme. A reversible inhibitor can be bound and released repeatedly: it's in equilibrium
- +2 To distinguish the two, the enzyme-inhibitor mixture can be dialyzed or run through a spin column to remove small molecules. +2 If the enzyme recovers its catalytic activity, inhibition was reversible. If it doesn't, it was irreversible.

(c; 7 pts) Sketch the Lineweaver-Burke plots corresponding to competitive and noncompetitive inhibition (two plots). Why does irreversible inhibition have the same kinetic profile as noncompetitive inhibition?

Plots: See Figs. 8.20 (competitive) and 8.22 (noncompetitive) on p. 228 of the textbook. In both, the line for no inhibitor is lower than the line in the presence of inhibitor. The two lines intersect on the Y-axis for competitive inhibition, and on the X-axis for noncompetitive. (4 pts total)

+3 In each case V_{max} is decreased and K_m remains the same. The underlying reasons are different: in the case of non-competitive inhibition, the EI complex forms equally well in the presence and absence of S (i.e., EI and EIS are both formed). So some enzyme is always in the inactive EI or EIS form regardless of the substrate concentration. In the case of the irreversible inhibitor, some enzyme has reacted with the inhibitor and is completely inactive, while the remaining enzyme is unaffected. The effect is the same as simply having less enzyme in solution. Thus the V_{max} is reduced but the K_m of the enzymes that are active is the same as that of uninhibited enzyme.

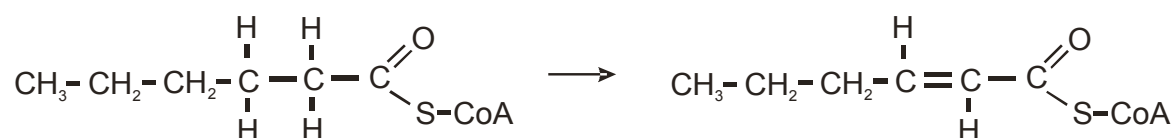
4. (30 pts) Metabolism and Glycolysis

(a; 8 pts) Six basic types of reaction that occur throughout metabolism are given in the textbook:

- | | |
|------------------------------------|--|
| 1. Oxidation-reduction | 4. Group transfer |
| 2. Ligation requiring ATP cleavage | 5. Hydrolytic |
| 3. Isomerization | 6. Addition/removal of functional groups |

Examine each of the reactions given below and identify the reaction type that best describes the reaction shown. (Note – the complete, balanced reaction is not necessarily shown!)

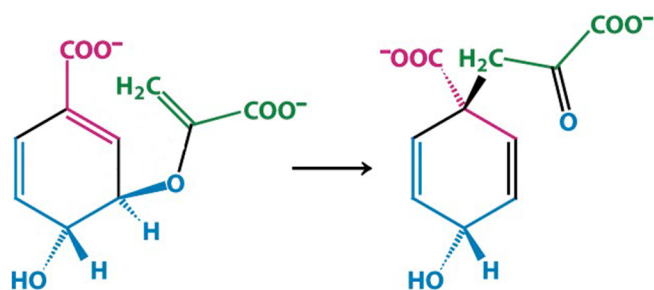
i) Reaction type: 1 Oxidation/reduction

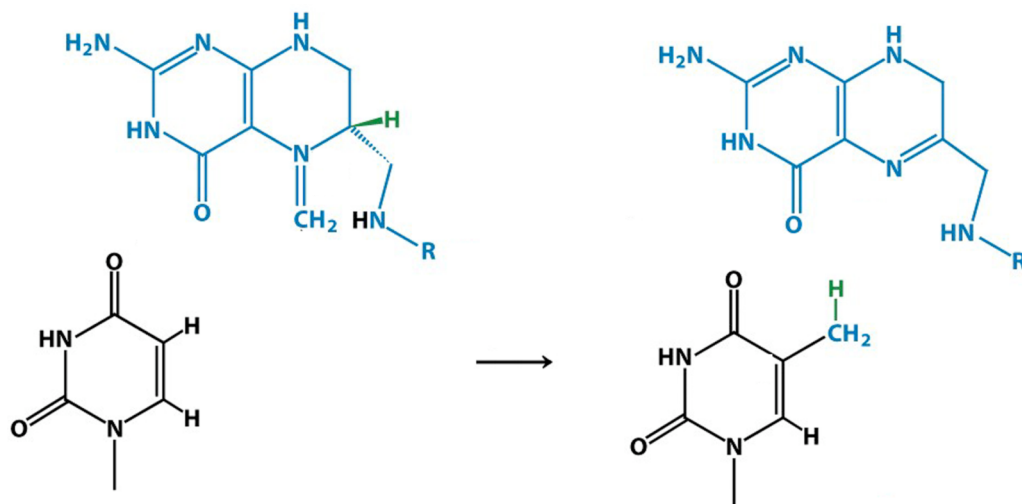


ii) Reaction type: 6 Removal of functional group



iii) Reaction type: 3 Isomerization



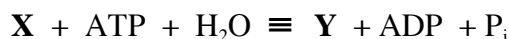
iv) Reaction type: 4 Group Transfer


(b; 12 pts) Consider the following simple chemical reaction:


 i) Calculate the value of $[Y]/[X]$ at equilibrium. Show your work. (The temperature is 25 °C (298 K), and remember that $R = 0.0083 \text{ kJ/mol/K}$.)

 The ratio $[Y]/[X]$ is just the K_{eq} for the reaction, so you must simply calculate K_{eq} :

$$K_{eq} = e^{-(\Delta G^\circ_N/RT)} = e^{-(20)/(0.0083 \cdot 298)} = 3.08 \times 10^{-4} = [Y]/[X]$$

 ii) Suppose now that X and Y participate in a sequence of reactions coupled to ATP hydrolysis:


For the following concentrations at equilibrium:

$$[\text{ATP}] = 2.25 \text{ mM} \quad [\text{ADP}] = 0.25 \text{ mM} \quad [\text{P}_i] = 1.65 \text{ mM}$$

 what is the ratio $[Y]/[X]$ at equilibrium for this reaction? Show your work. (Remember that $\Delta G^\circ_N = -30.5 \text{ kJ/mol}$ for ATP hydrolysis).

$$\text{For this reaction, } K_{eq} = [Y][\text{ADP}][\text{P}_i] / [X][\text{ATP}] \quad [Y]/[X] = K_{eq} \cdot [\text{ATP}] / [\text{ADP}][\text{P}_i]$$

 First calculate ΔG°_N and K_{eq} for this reaction. The ΔG°_N is the sum of that for $X \rightleftharpoons Y$ plus that for ATP hydrolysis: $\Delta G^\circ_N = +20 + (-30.5) = -10.5 \text{ kJ/mol}$

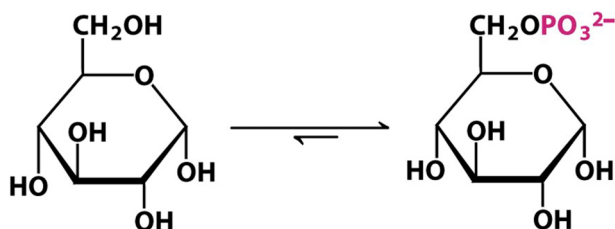
$$K_{eq} = e^{-(\Delta G^\circ_N/RT)} = e^{-(-10.5)/(0.0083 \cdot 298)} = 69.8$$

$$[Y]/[X] = (69.8)(2.25 \times 10^{-3}) / (0.25 \times 10^{-3})(1.65 \times 10^{-3}) = 3.8 \times 10^5$$

(c; 10 pts) Shown below are three of the reactions from **glycolysis**. For each reaction:

- 1) write the name of the enzyme that catalyzes the reaction.
- 2) write the name of the reactant(s) shown.
- 3) list any additional reactants and/or products that are not shown.

i)

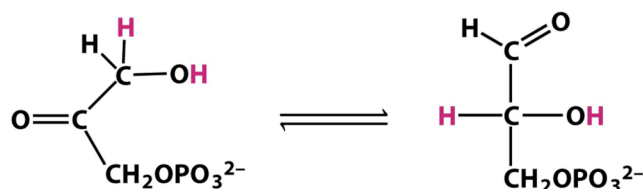


enzyme: **hexokinase**

reactant: **glucose**

other reactants/products: **ATP, ADP**

ii)

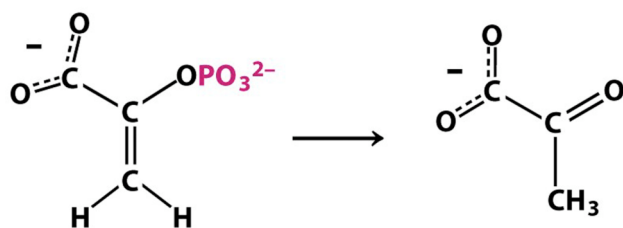


enzyme: **triosephosphate isomerase**

reactant: **dihydroxyacetone phosphate**

other reactants/products: **none**

iii)



enzyme: **pyruvate kinase**

reactant: **phosphoenolpyruvate (PEP)**

other reactants/products: **ATP, ADP**

Score for the page _____

