

You have 120 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 and schemes are given on a tear-off sheet at the end.

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. Thermodynamics (50 pts)

(a; 12 pts) In the table below, fill in the signs of ΔG° and whether the reaction is spontaneous at all T, spontaneous at no T, spontaneous at low T, or spontaneous at high T. (Don't be insulted, some of the questions below are harder.)

Sign of ΔH°	Sign of ΔS°	Sign of ΔG°	Spontaneity: circle none, one, or both
+	+	+ at low T - at high T	low T high T
+	- <i>+1</i>	+ at low T + at high T	low T high T
-	+ <i>each</i>	- at low T - at high T	low T high T
-	- <i>for total +8</i>	- at low T + at high T	low T high T

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

(b; 4 pts) Give an example of an exothermic ordering reaction.

DNA hybridization
 Water crystallization \rightarrow ice
 Fetal development

+4 for any correct answer

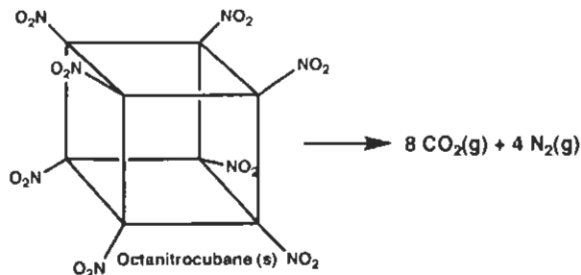
(+2 for exothermic, if wrong, +2 for exothermic or ordering)

(c; 4 pts) Give an example of an endothermic disordering reaction.

DNA melting
 Ice melting
 Urea dissolving in water
 N_2O_4 dissociation to $2NO_2$

+4 : +2 each for endothermic + $\Delta S > 0$

(d; 8 pts) What are the signs of ΔS° and ΔH° for the reaction at the right, and why?



+1 $\Delta S^\circ > 0 \rightarrow$ we are going from one molecule of solid to

+2 12 molecules of gas \rightarrow large increase in vanderwaals motion

+1 $\Delta H^\circ < 0 \rightarrow$ strongly exothermic - we are breaking relatively

+2 for either $\left[\begin{array}{l} \text{weak N-O bonds, and the molecule has a lot of} \\ \text{strain energy, to form very stable products +1} \\ \text{CO}_2 \text{ and N}_2 \end{array} \right. \quad (\Sigma = 9, \text{ total of 8 available})$

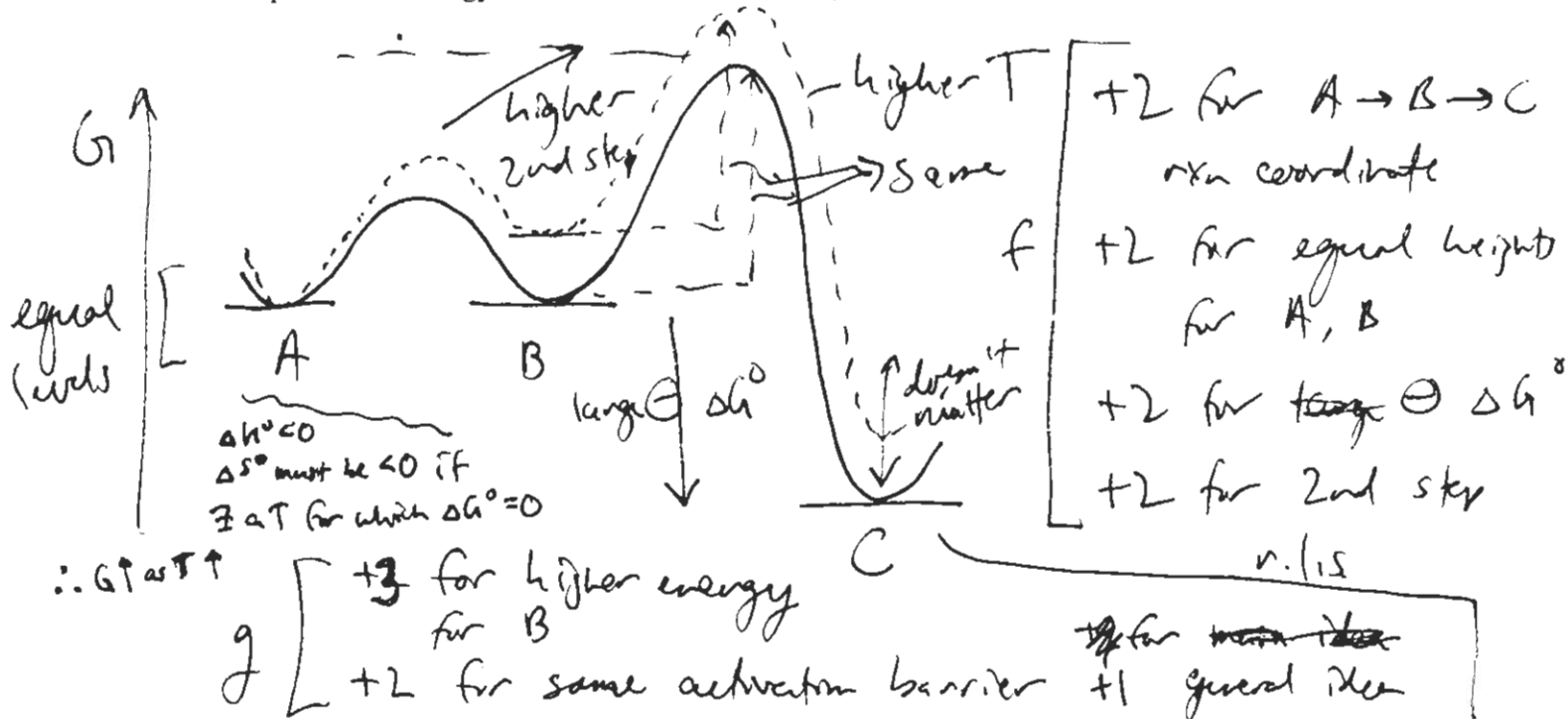
(e; 2 pts) What does part (d) tell us about the kinetics of the octanitrocubane decomposition reaction? (A one-word answer will do.)

+2 nothing \equiv just that $\frac{k_{\text{for}}}{k_{\text{rev}}} \gg 1$

Consider the reaction $A \leftrightarrow B \rightarrow C$, where (1) the overall reaction $A \rightarrow C$ is strongly downhill, (2) the reaction is a rapid pre-equilibrium followed by a rate-limiting step, (3) the equilibrium $A \leftrightarrow B$ is exothermic, and (d) the activation free energy for the $B \rightarrow C$ step is temperature-independent.

(f; 8 pts) In the space below, draw a free energy reaction coordinate diagram for the reaction, at a temperature at which $K_{eq} = 1$ for $A \leftrightarrow B$. $A \leftrightarrow B \rightarrow C$

(g; 6 pts) On the same diagram, draw the curve observed at a higher temperature; be sure to label your curves. Assume that the free energy of the reactant A is constant (in practice, it doesn't matter -- we adopt the free energy of A as the reference state).



(h; 6 pts) With reference to your diagram, explain why we might observe an apparent negative activation free energy for the overall reaction $A \rightarrow C$ using the standard Eyring equation to measure ΔG^\ddagger .

~~$\ln k = \ln A - \frac{\Delta G^\ddagger}{RT}$~~

$k = A e^{-\Delta G^\ddagger / RT}$ $\ln k = \left(-\frac{\Delta G^\ddagger}{R} \right) \cdot \frac{1}{T} + \ln A$

As $T \uparrow$ we expect $\ln k$ to \uparrow if ΔG^\ddagger is > 0

- From the diagram, we see that the overall ΔG^\ddagger gets

+3 | more \oplus as $T \uparrow \rightarrow$ i.e. the reaction may get slower! This would end up giving us a $\ominus \Delta G^\ddagger$.

On full credit for explanation in terms of a \downarrow [B] leading to slower production of C.

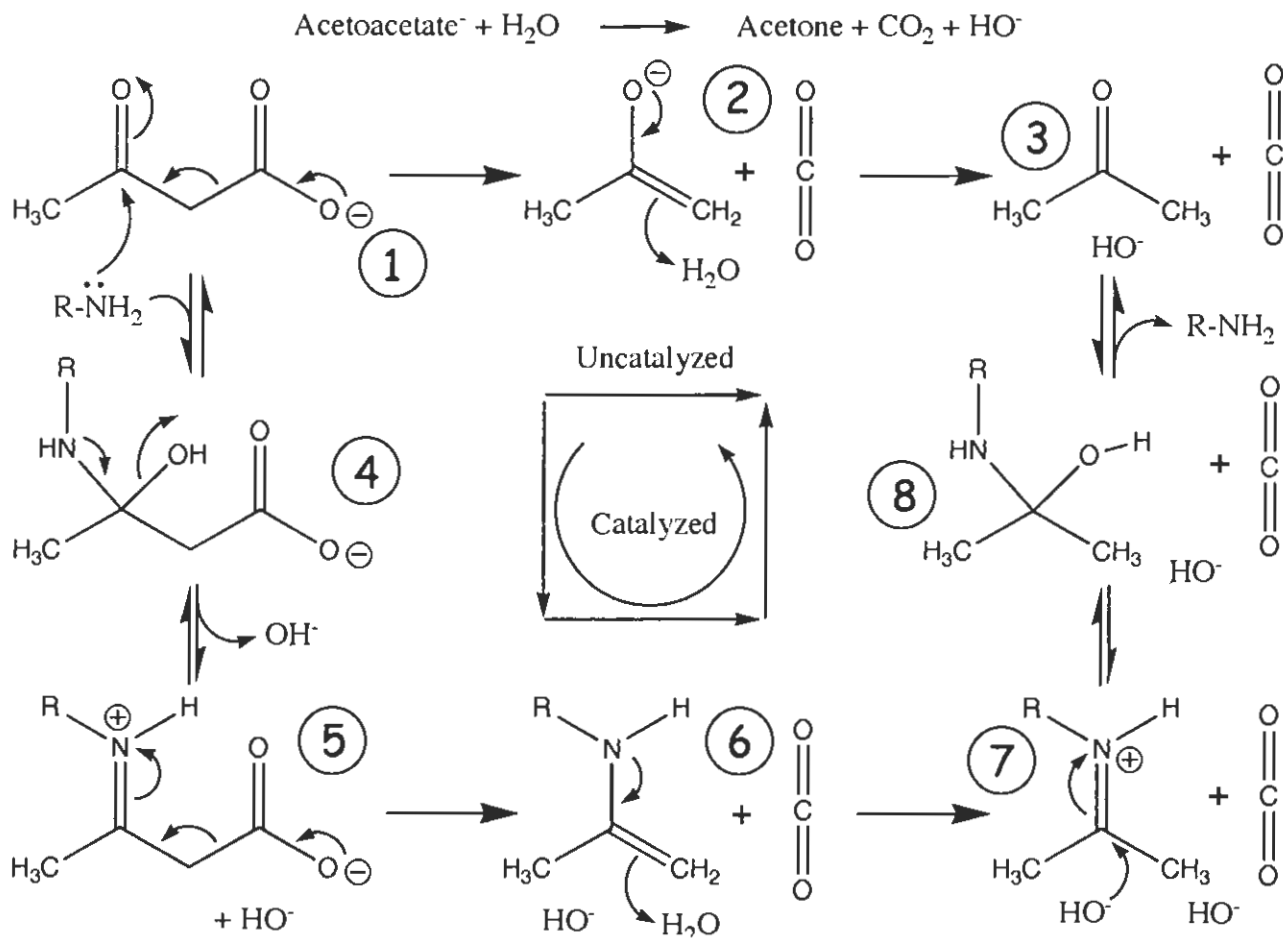
+3 for any justification in terms of $k = A e^{-E_a/RT}$

2. Acetoacetate (95 pts)

This is an extended question covering many aspects of the chemistry and biochemistry of acetoacetate. You can answer many of these questions even if you have missed those that came before. This is my idea of an integrated question, one that demonstrates that a competent chemist picks up whatever theoretical or experimental tool is appropriate to the problem at hand.

Acetoacetate is an intermediate in amino acid catabolism, and it is produced from fat in the bodies of people who are starved for glucose, or whose bodies think they are starved (diabetics). Its decarboxylation is responsible for the smell of acetone on the breath of patients with uncontrolled diabetes. Acetoacetate is oxidized to provide energy in the brains of starving people.

We are concerned first with the mechanism of acetoacetate decarboxylation, both for the uncatalyzed reaction shown along the top of the scheme below and also the reaction catalyzed by primary amines along the bottom three sides of the square. The pH is roughly 7. For your reference, the same scheme is reprinted on the last page of the exam.



(a; 6 pts) Is there net oxidation or reduction of carbon atoms in this reaction? What is the formal oxidation state of each of the four carbons in acetone and CO₂?

+2 No net redox → all the H, O stay the same, so so do C.

$$\begin{array}{c}
 \text{CH}_3 \\
 | \\
 \text{C} \\
 | \\
 \text{CH}_3
 \end{array}
 + \begin{array}{c}
 \text{O} \\
 || \\
 \text{C} \\
 || \\
 \text{O}
 \end{array}$$

↓ ↓ ↓ ↓

-3 +2 -3 +4

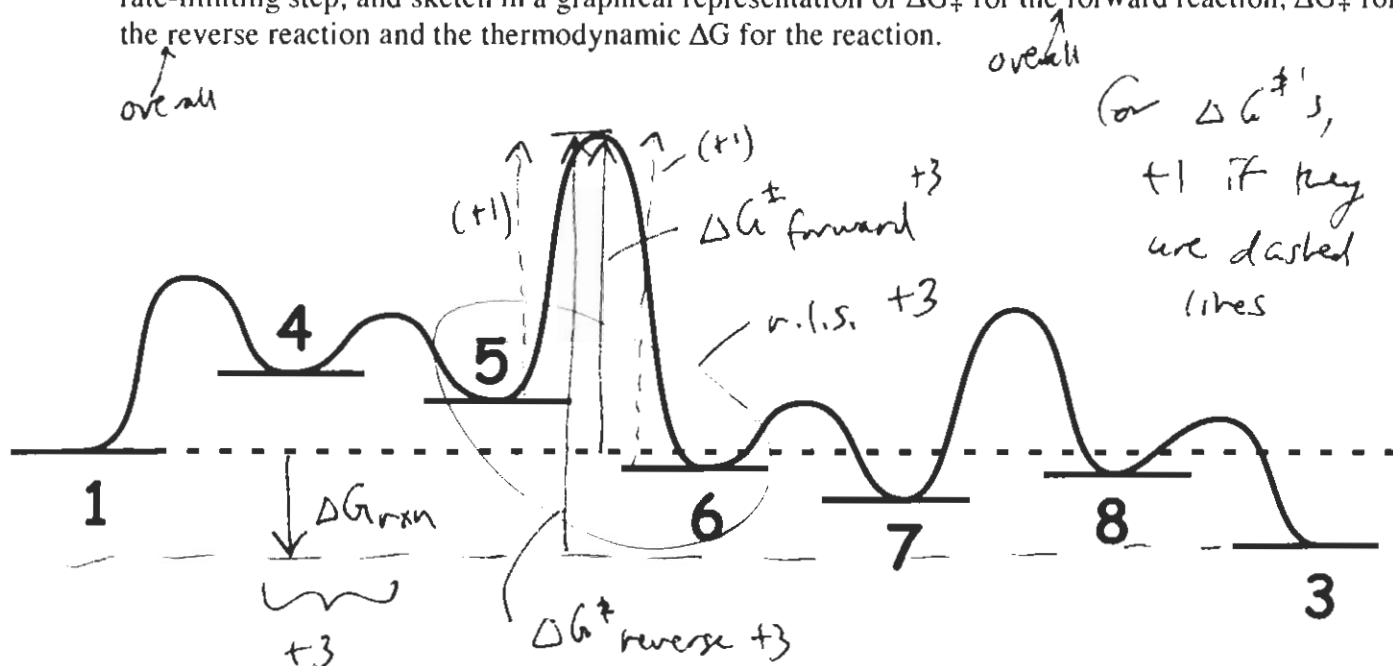
Σ = 0

$$\begin{array}{c}
 \text{H}_3\text{C}-\overset{\text{R}}{\text{C}}-\text{CH}_2-\overset{\text{R}}{\text{C}}-\text{O}^- \\
 \uparrow \quad \uparrow \quad \uparrow \quad \uparrow \\
 -3 \quad +2 \quad -2 \quad +3
 \end{array}$$

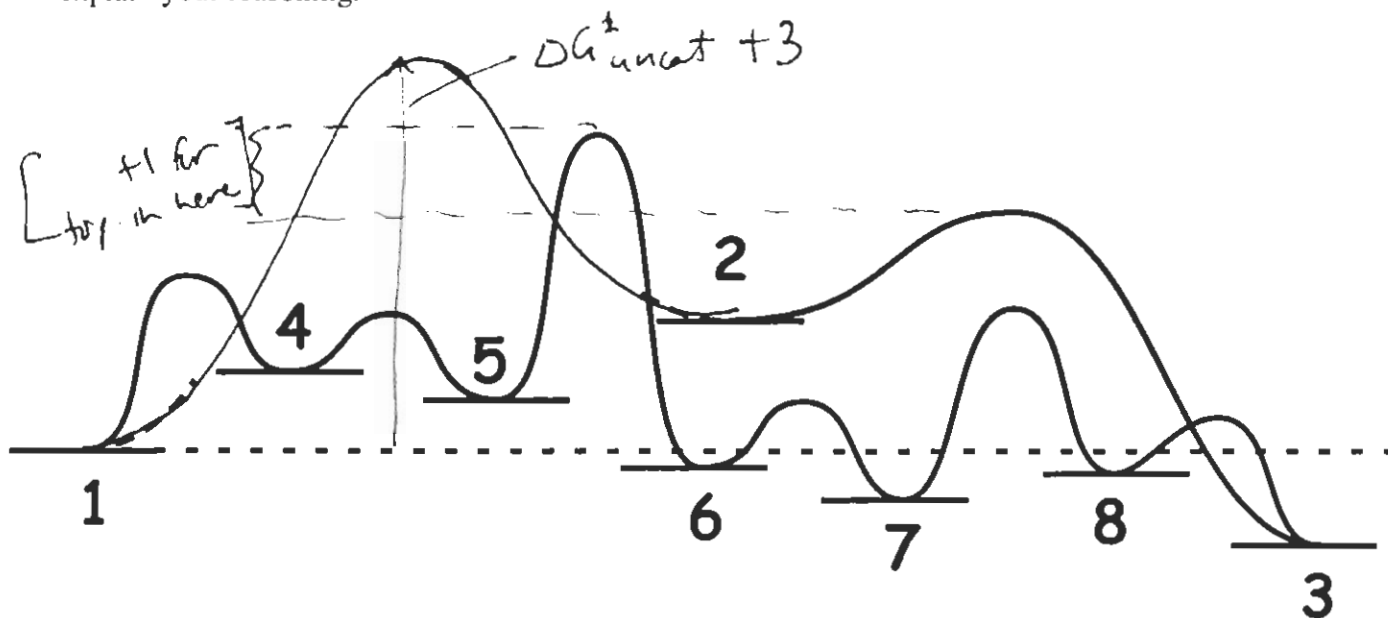
Σ = 0 - same as carbohydrate

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(b; 12 pts) On the free energy reaction coordinate diagram below for the catalyzed reaction, identify the rate-limiting step, and sketch in a graphical representation of ΔG^\ddagger for the forward reaction, ΔG^\ddagger for the reverse reaction and the thermodynamic ΔG for the reaction.



(c; 9 pts) The rate-limiting step of the uncatalyzed reaction is the same chemical transformation as for the catalyzed reaction, at least around pH 7. Given this fact, on the diagram below sketch in the remainder of the free energy reaction coordinate diagram for the uncatalyzed reaction. Briefly explain your reasoning.



- +3 - Decarboxylation is r.l.s. → barrier must be higher than barrier for 2nd step / OK if missing it since curve is
- +3 - Rxn is catalyzed - barrier must be higher than ΔG^\ddagger for the catalyzed rxn

The catalyzed mechanism shown above resembles that of the enzyme acetoacetate decarboxylase, which is produced by the obligate anaerobic bacterium *Clostridium acetobutylicum* shown at the right. This bug produces acetoacetate as one product of carbohydrate metabolism, and then degrades it using acetoacetate decarboxylase in order to raise its intracellular pH. There is renewed interest in this "solventogenic" fermentation as a means of industrial production of acetone. Accompanying reactions give butanol and ethanol, so this is called ABE fermentation. Cars can run on "biobutanol."



(d; 3 pts) How does the overall reaction function to neutralize the cytoplasm of *Clostridium*?

should have included this over again / $acetoacetate^- + H_2O \rightarrow acetone + CO_2 + HO^-$ (+2)
 the HO^- raises the pH. Works because CO_2 can escape rather than \rightarrow carbonate - or you could
 (or - removal of acidic acetate OK +2)
 acetate decarboxylase argues that carbonate is a weaker acid

(e; 4 pts) Would the free energy change for the reaction become more negative or more positive as pH decreases? Why?

As pH \downarrow , HO^- \downarrow , therefore rxn is pulled to the right - ΔG becomes more \ominus . (+2)
 (or H^+ reacts with HO^-) (+2)

The mechanism doesn't matter, only reactants + products!

(f; 8 pts) Fermentation was referred to in class as a means of living via rearranging the deck chairs. What did this mean in terms of redox chemistry? *Clostridium* is poisoned by oxygen, but even if it weren't it couldn't compete with aerobic bacteria living on carbohydrates. Why not?

+4 | - In fermentation there is ~~not~~ no net oxidation/reduction of fuel, only rearranging of carbon skeleton. (+3)
 Must balance the electrons for a fermentation \rightarrow this greatly decreases the available free energy. (+1)

+4 | - The aerobically growing bug could oxidize carbohydrates all the way to CO_2 instead of leaving ABE fuel on the table - can

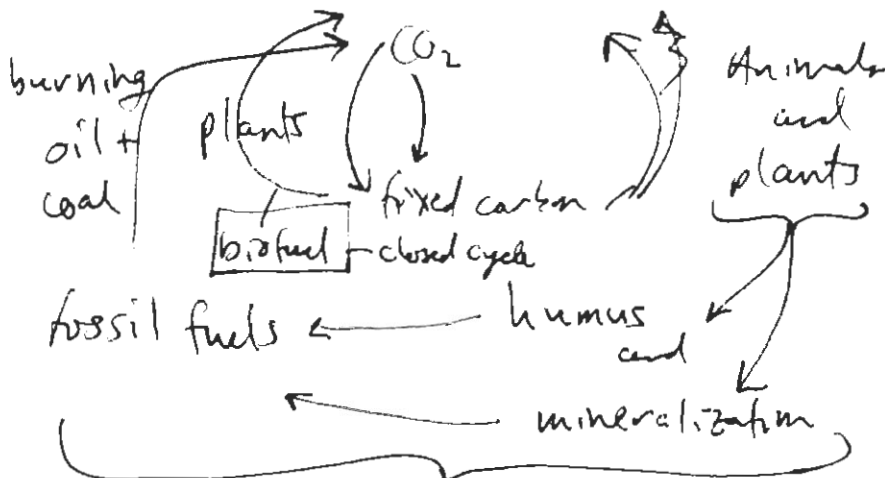
(+2) [harvest a lot more free energy (or can do this "using O_2 as terminal acceptor")]

(g; 6 pts) Briefly, what is the thermodynamic origin of the stratification of nitrate reducers and sulfate reducers in the Black Sea?

+3 for description of stratification

High up in the water column, aerobic bacteria deplete O_2 . Below this level there is a niche for bugs that transfer e^- from carbohydrates to NO_3^- - they can obtain nearly as much free energy as an aerobic. When NO_3^- is depleted, only then can SO_4^{2-} ~~provide~~ ^{reducers obtain} free energy to live - above that level they can't compete. [+2 for just realizing that O_2 is the best oxidant]

(h; 8 pts) With reference to the global carbon cycle, explain why biofuel (like biobutanol produced as above) may be better than fossil fuel in terms of climate change. (Provided that it doesn't take too much fossil fuel energy to process the biofuel...this is one big problem with corn-based ethanol.)



biofuels simply recycle CO_2 into fuel and re-burn it - ~~not~~ no net increase in CO_2 .

+3 for idea of the cycle or if implicit

Fossil fuels add CO_2 to the atmosphere →

[+2 for concept of renewable energy]

+2 | ↑ green house warming

[+2 for "release less CO_2 "]

(i; 8 pts) The catalytic R-NH₂ group in the acetoacetate decarboxylase enzyme is an active site lysine. The pK_a of the ε-NH₃⁺ group is around 6, much lower than that of the free amino acid. In general, why does burial of a lysine in the interior of a protein tend to cause a drop in its pK_a? This explanation doesn't work as well in the context of a solvent-exposed active site: what other protein structural element could cause a decrease in pK_a in this situation?

- Burial of charge in a hydrophobic environment ⁽⁺²⁾ is thermodynamically unfavorable - the decrease in pK_a means that Lys is more likely to be neutral. ⁽⁺³⁾

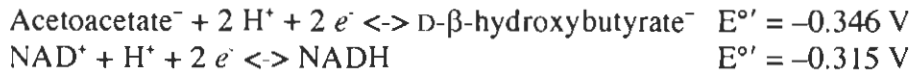
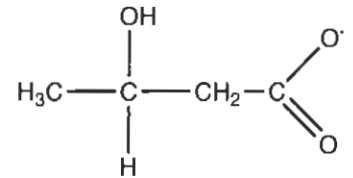
- What else could cause a decrease in pK_a? proximity of another + charge ⁽⁺³⁾ from Lys or Arg will destabilize + charge on the active site Lys → promote loss of H⁺.

give credit in either place

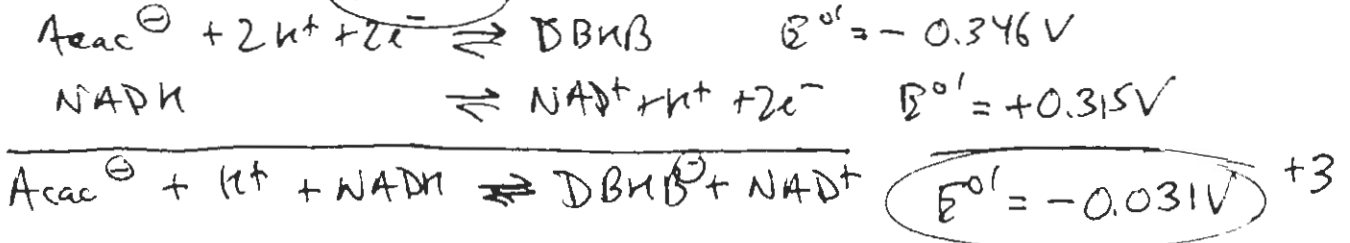
(j; 6 pts) The enzyme has a pH optimum around 6. According to the mechanism given, why does the rate drop off markedly as the pH decreases? (Note that this implies a change in which step is rate limiting.)

The R-NH₂ must be deprotonated ⁽⁺³⁾ in order to ⁽⁺³⁾ act as a nucleophile in the first step, formation of the carbandamide. At low pH this step will be much slower b/c the lysine will be protonated - first step becomes r.l.s. - ~~the rate is not affected~~

Under some conditions, acetoacetate can be reduced by NADH to give D-β-hydroxybutyrate (at right), with the half reactions and standard reduction potentials shown below.



(k; 9 pts) Calculate E°' and ΔG°' and show that K_{eq} = 0.089 at pH 7 for the reduction of acetoacetate. A+25°C



$$\Delta G^{\circ'} = -n F E^{\circ'} = -2 \cdot 96500 \text{ C/mole} \cdot (-0.031\text{V}) = +5983 \text{ J/mole}$$

$$K_{eq} = e^{-\Delta G^{\circ'}/RT} = e^{-5983 / (8.314 \cdot 298)} = 0.089$$

(+1) (+1) (+2) (+1) (+1) for consistent

(l; 16 pts) What is the equilibrium constant expression for this reduction? We will use the abbreviations BHB and Acac for brevity. If the ratio of [NAD⁺]/[NADH] = 1, pH = 7, and [Acac] = 2 mM, what is [BHB]? If [NAD⁺]/[NADH] = 1000 and [Acac] is 3 mM, what is [BHB]? Why have biochemists studying rat liver used an elevated ratio of [D-β-hydroxybutyrate⁻]/[acetoacetate⁻] as a sign of hypoxia (lack of O₂)?

$$K_{eq} = \frac{[\text{BHB}][\text{NAD}^+]}{[\text{Acac}][\text{H}^+][\text{NADH}]} \quad +3$$

Standard state (+1) for recognizing this

$$[\text{BHB}] = K_{eq} \cdot [\text{Acac}] \cdot \frac{[\text{NADH}]}{[\text{NAD}^+]} = 0.089 \times 2\text{mM} = 0.178\text{mM} \quad +2$$

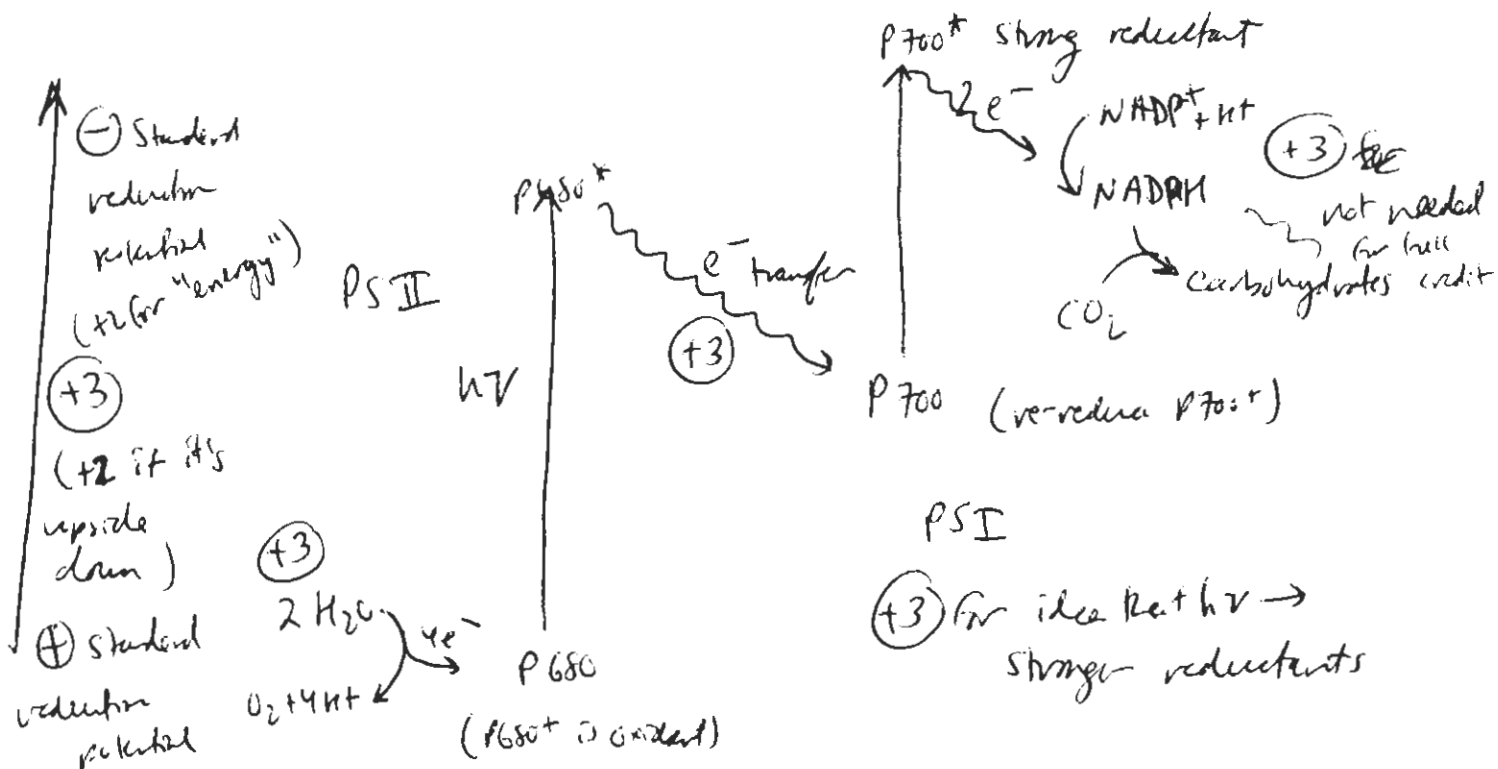
if [NADH]/[NAD⁺] = 1/1000, have [BHB] = $0.089 \times 3 \times 10^{-3} \text{m} \times \frac{1}{1000} = 2.67 \times 10^{-7} \text{m}$ ~~1.78 x 10⁻⁴ m~~
 (+2) for setup consistent w/ above

If ∃ hypoxia, NADH will not be re-oxidized +3

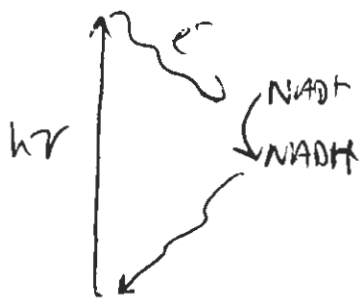
Therefore [BHB]/[Acac] is elevated b/c [NADH]/[NAD⁺] is high.
 (+3) (OK if any (2pt))

3. Redox Reactions to Live By (25 pts)

(a; 15 pts) Sketch the Z scheme for photosynthesis. You do not need to write down the names of intermediate electron acceptors. Specify what the y-axis represents, but you do not need to know the numerical values on it. *donors and*



(b; 10 pts) Some photosynthetic bacteria lack Photosystem II (the left half of the Z scheme), and the electron donated by the PS I chromophore eventually returns to it. Obviously, these bugs can't use those electrons to fix CO₂ the way plants do. By analogy with oxidative phosphorylation, what benefit do they derive from this cyclic electron transfer?



The transfer of e⁻ from NADPH back to the oxidized chromophore is strongly downhill.

They use this driving force to pump protons or other ions, and then tap the gradient to make ATP. [Plants do this too!]

Idea of the cycle - +2 or +3
 Using NADPH reoxidation for ΔG - +5
 "dark energy" is +2 of 5

gradient +3
 make ATP +2

Score for the page _____

4. Kinetics (30 pts)

This question has not changed significantly from Exam II except I have added part (g).

Kinetic partitioning describes the distribution of products for reactions under kinetic control, such as correct and incorrect base incorporation by DNA polymerases.

For the elementary reaction $A \rightarrow B$, the differential rate law is $-d[A]/dt = k[A]$.

(a; 3 pts) What is the integrated rate law for a the first-order reaction $A \rightarrow B$ assuming that we start with 100 % A at concentration $[A]_0$ (not a trick question)?

$$[A] = [A]_0 e^{-kt} \quad (+3)$$

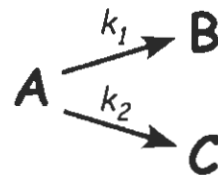
(b; 6 pts) Given that all of the starting material that starts out as A can always be found as either A or B at any time during the reaction, derive an integrated rate law expression for the amount of B as a function of time.

$$[A] + [B] = [A]_0 \quad (+2) \quad (+2) \text{ for idea of substituting}$$

$$[B] = [A]_0 - [A]_0 e^{-kt} \quad (+2) \text{ for either}$$

$$= [A]_0 (1 - e^{-kt})$$

(c; 6 pts) Now consider the scheme at the right, where A can be converted to either B or C.



What are the differential rate laws for loss of [A], gain of [B], and gain of [C]?

Remember, k_1 and k_2 just indicate which reaction is which. The subscripts do not refer to reaction order.

$$-\frac{d[A]}{dt} = k_1 [A] + k_2 [A] = (k_1 + k_2) [A] \quad +2$$

$$\frac{d[B]}{dt} = k_1 [A] \quad +1$$

$$\frac{d[C]}{dt} = k_2 [A] \quad +1$$

+2 for idea of breakdown + production (i.e. correct signs)

(d: 3 pts) B and C are always produced at a constant ratio (in other words, for every mole of B we get x moles of C. From your answer to (c), what is $[C]/[B] = x$?

$$\begin{aligned} [B] &\propto k_1 [A] & \frac{[C]}{[B]} &= x = \frac{k_2}{k_1} & +3 \\ [C] &\propto k_2 [A] \end{aligned}$$

(e: 3 pts) By analogy with your answer in (a) for $A \rightarrow B$, what is the integrated rate law for the concentration of A as a function of time?

$$[A] = [A]_0 e^{-(k_1 + k_2)t} \quad +3$$

(f: 6 pts) The total concentration of products is given by $[B] + [C] = [A]_0 - [A]$ as in (b) above. Use your answers to (d) and (e) to derive the integrated rate law for the total amount of [B] as a function of time.

$$[B] + [C] = [A]_0 - [A]$$

Substitute (+2 each)

$$[B] + \frac{k_2}{k_1} [B] = [A]_0 - [A]_0 e^{-(k_1 + k_2)t}$$

$$[B] \left(1 + \frac{k_2}{k_1} \right) = [A]_0 (1 - e^{-(k_1 + k_2)t})$$

$$[B] = \frac{k_1}{k_1 + k_2} [A]_0 (1 - e^{-(k_1 + k_2)t}) \quad (+2)$$

(g; 3 pts) What is $t_{1/2}$ for this reaction?

$t_{1/2}$ is the same for loss of [A] and production of [B] and [C]

$$t_{1/2} = \frac{\ln 2}{k_1 + k_2} \quad (+3) \quad (+1 \text{ for any attempt})$$

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