Final Exam Review Session

April 19th

12:00 pm

Question 1

The enzyme carbonic anhydrase of erythrocytes catalyzes the hydration of CO2:

This reaction is important in the transport of CO2 from the tissues to the lungs.

Carbonic anhydrase is a monomeric enzyme with a molecular mass of 30, 000 Daltons.

Question 1

(a) Provide the 1 word (semi) systematic name for the enzyme _____ (2 points).

Synthase:

- Joins 2 molecules into 1
-H_2O can be one of the molecules! (KEY: In reverse reaction, H_2O does not become a part of both molecules)
- Can be called lyase (reverse)

Hydrolase:

-1 molecule cleaved into 2 molecules by H_2O.
-Part of H_2O in both molecules

Synthetase:

-Joins to molecules into 1, using ATP.
-Usually has enzyme bound intermediates
-Can be called ligase (reverse)

Isomerase:

-Functional groups move around in 1 molecule

Transferase:

-Part of one molecule is moved to another molecule
-**Kinase:** moves phosphate from ATP to another molecule

Oxidoreductase:

-Oxidation-reduction reactions between two molecules

Dehydrogenase:

-Oxidation-reduction reaction with 2e- and 1 or 2 H+
-Usually involves NAD+/NADH

Question 1

(a) Provide the 1 word (semi) systematic name for the enzyme Synthase **(2 points).**

(b) (2 points) The kcat for the enzyme (pH = 7.4) is 1.5 x 105 sec-1. State in a single sentence what a kcat value of 1.5 x 105 sec-1 means.

General Definition:

Amount of reactant that **one molecule of enzyme turnsovers to product** every **second** when all **100% of active sites** are **filled.**

Any Questions on Kcat?

Question 1

(c) A series of enzyme assays was carried out with an unknown quantity of carbonic anhydrase. The data was subjected to a Lineweaver-Burk analysis and the following results were obtained

Slope of line, m = 1.5 min/L. = km/vmax

Intercept on y-axis, b = 0.17 (mmol/min)-1 = 1/vmax

- make sure to keep track of units!
(ei. mmoles or moles... Choose one or the other)

(i) (5 points) What quantity of carbonic anhydrase (in ug) was used in the enzyme assays? **19.5 ug**

What variable has the amount of enzyme in it? kcat

Kcat = vmax/ amount of enzyme

Also 30,000 Da = 30,000 g/mol

Question 1

ii) (2 points) Calculate Km. Express your answer in mM.

Get from slope given → slope = km/vmax

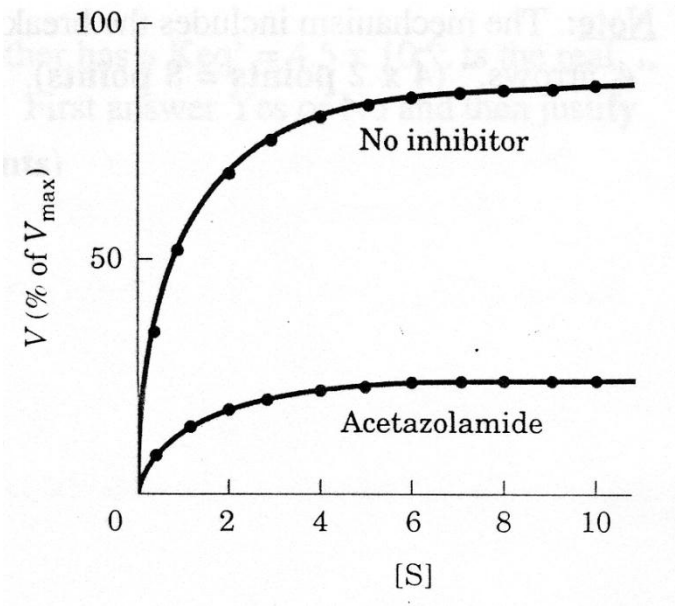
8.8 mM

(iii) (2 points) Using the concepts of active site filling and maximum reaction rate, explain in 2-3 sentences what a Km value represents.

The amount of substrate needed to get half of the maximum rate, which is the same as 50% of the active sites being filled.

Question 1

(d) Carbonic anhydrase is inhibited by acetazolamide, a drug used to treat glaucoma (high pressure within the eyeball). The experimental curve of reaction velocity, given here as percentage of Vmax versus [S, units not shown] for the carbonic anhydrase reaction (+/-acetazolamide) is illustrated (**Note:** the same enzyme concentration was used in both cases).

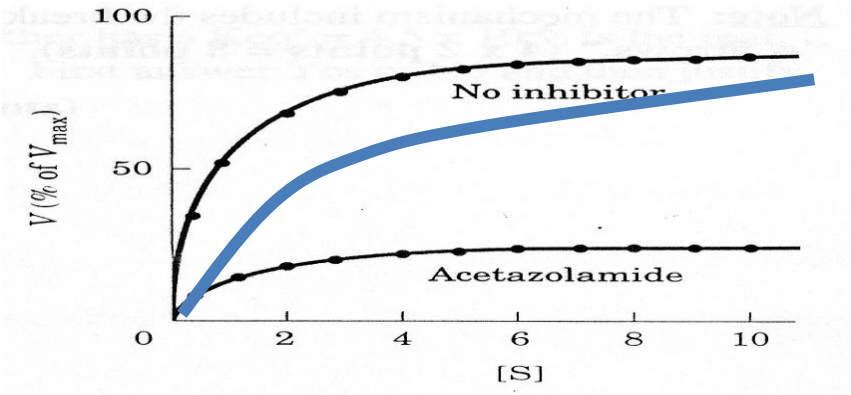


i. **(1 point) Is the data consistent with the idea that the inhibitor reversibly binds in the active site**

Yes or No? **(Circle one)**

ii. **(4 points) Briefly explain your answer to (i). Begin your answer in the space next to the graph.**

What should the graph look like?



Question 2

(ii) (4 points) Briefly explain your answer to (i). Begin your answer in the space next to the graph.

(a) (5 points) Calculate the threshold amount of energy that must be supplied to synthesize 1 mol of glycylglycine by the following mechanism .

Assume that the following concentrations of products and reactants are maintained at the following steady state levels during the reaction in the cell:

[Glycine] = 5 mM [Glycylglycine] = 50 μM [H_2O] = 55.5 M

Do we use this?

No, look at page 121 in notebook. H2O and H+ assumptions!

+ 5.7 kJ/mol

Question 2

(b) If the hydrolysis of ATP to AMP and PPi is going to supply the energy to synthesize 1 mol of glycylglycine in (a) the value of Qcell' value for the ATP hydrolysis cannot equal or exceed a certain value. Calculate this value. (5 points).

$$ATP + H_2O \longrightarrow AMP + PPi + H^+ \qquad \Delta G^{o'} = -31.8 \text{ kJ/mol}$$

What values do we use?

Want to know the bare minimum Q cell of ATP to AMP hydrolysis, so we use:

The energy needed to synthesize glycylglycine

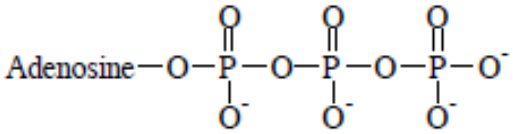
Which Keq do we use? Derive from

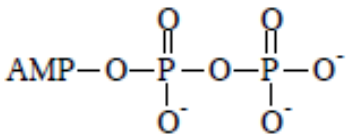
$$\Delta G = -kB \cdot N \cdot T \cdot \ln (Keq/Qcell)$$

Qcell' cannot equal or exceed 3.78×10^4

Question 2

(c) Provide an “arrow-pushing” mechanism for the real synthetase catalyzed reaction based on the mechanisms of other anabolic reactions. Note: the mechanisms presented in class did not show any enzyme active site residues nor did they keep track of protons (H+). You can use the following symbols for ATP also used in class:





Note: To indicate the mechanism use only 4 arrows. Do not include arrows for rearrangements indicating the changes in geometry around P (Step #1) or C (Step #2) (2 x 4 points = 8 points).

-Show:

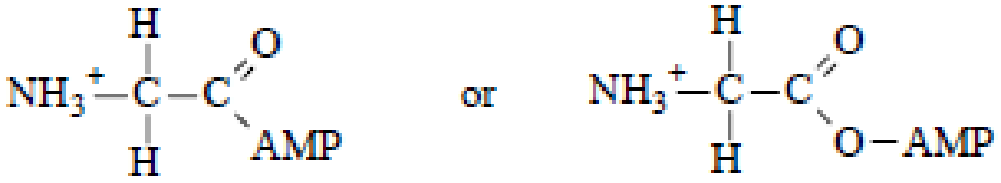
- Nucleophilic Attacks
- Leaving Groups

-DO NOT show rearrangement!

Keep in mind what is made (ei. Is ADP or AMP made?)

Question 2

(d) In your mechanism, indicate which molecule is the "high energy intermediate"(2 points)



Definition of a high energy intermediate:

-Imaginary hydrolysis produces energy greater than or equal to that of ATP hydrolysis

-Is always a intermediate step, not the end product

Question 3

In the laboratory artifical electron donors can be used to donate electrons to the ETC components. One such artificial electron donor is “hexaammineruthenium”

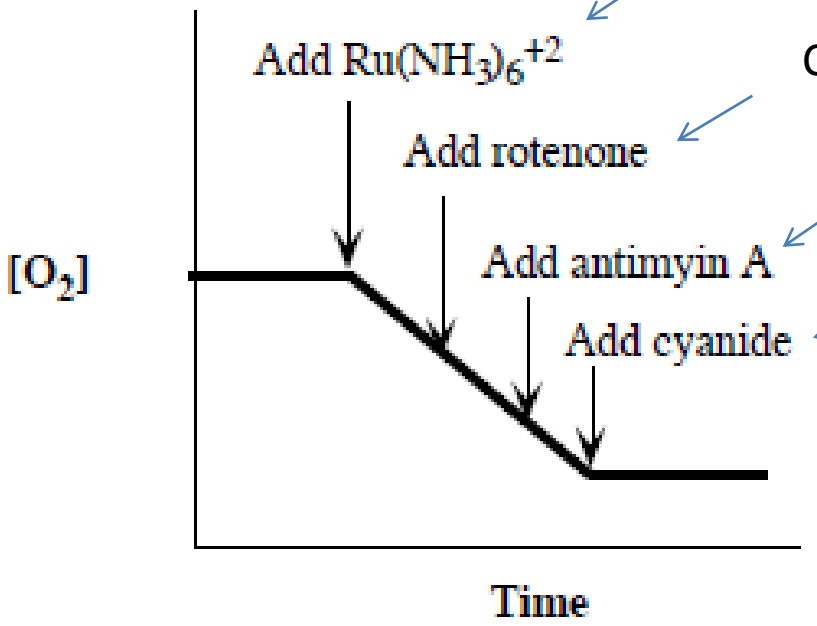
Reduced Form	Oxidized Form
$Ru(NH_3)_6^{+2}$	$Ru(NH_3)_6^{+3}$

(a) (2 points) The oxidized and reduced forms differ by **1** electron(s) and **0** proton(s).

Question 3

(b) In laboratory experiments, the following oxygen consumption curve was obtained for intact mitochondria incubated in the presence of $Ru(NH3)_6^{+2}$, O2, ADP and Pi.

(i) (2 points) Which two components of the ETC could be accepting electrons from $Ru(NH3)_6^{+2}$?



Add electrons to a complex in the ETC

Complex must be after complex I

Complex must be after complex III

Complex must be before 0_2 is reduced to H_2O

Cytochrome C or Complex IV

Question 3

(ii) (2 points) What is the expected P:O ratio in each case?

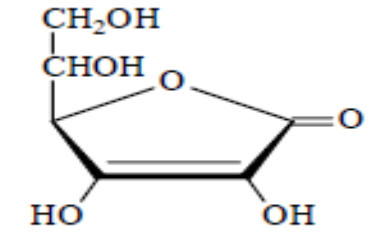
P:O if donating to Component #1 _____1:1_____

P:O if donating to Component #2 _____1:1_____

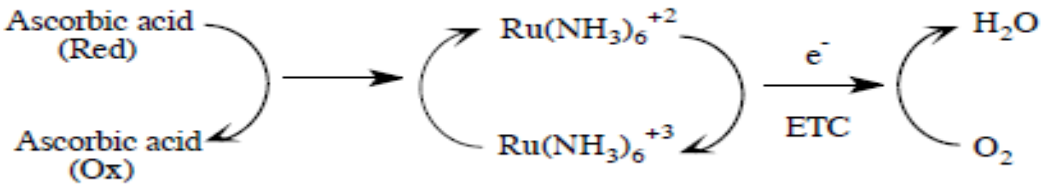
-Pg. 156: Follow the diagram
-4H needed to make 1 ATP

Question 3

c) In the laboratory, $Ru(NH3)_6^{+3}$ can be used to carry electrons from reduced ascorbic acid to the ETC ulimately to O_2



Reduced ascorbic acid



(i) (4 points) The equation for the oxidation of ascorbic by oxygen is

$$C_6H_8O_6 + 1/2O_2 \longrightarrow C_6H_6O_6 + H_2O \qquad Keq' = 4.4 \times 10^{25}$$

Assuming standard conditions for the oxidation of ascorbic acid and ATP synthesis, could the oxidation of 1 mol of ascorbic acid provide enough energy to synthesize 4 mol ATP? Support you answer with a calculation.

Yes → 146.2 kJ > 122 kJ

Question 3

(ii) (4 points) Oxidative phosphorylation is occurring in isolated mitochondria using ascorbic acid as electron donor. The mitochondria are suspended in a solution buffered at pH = 6.0.

If the electrical charge difference across the IMM is 0.18 V (inside negative) and the matrix pH = 7.0, calculate how much energy is produced when 3 mol of H+ enter the matrix via the ATPase.

$$\Delta G = -kB \cdot N \cdot T \cdot \ln (C2/C1) + (Z \cdot F \cdot \Psi)$$

$\Delta G' = 2476 \ln (10^{-7}/10^{-6}) + (+1)(96, 480)(-0.18) = -23.0$ kJ/mol H+

69 kJ energy produced

Rule for Ψ

If transport is favourable, then Ψ is negative

For example:

Positively Charged Species goes from **HIGH** positively charged are to a **LOW** positively charged area = favourable

If transport is unfavourable, then Ψ is positive

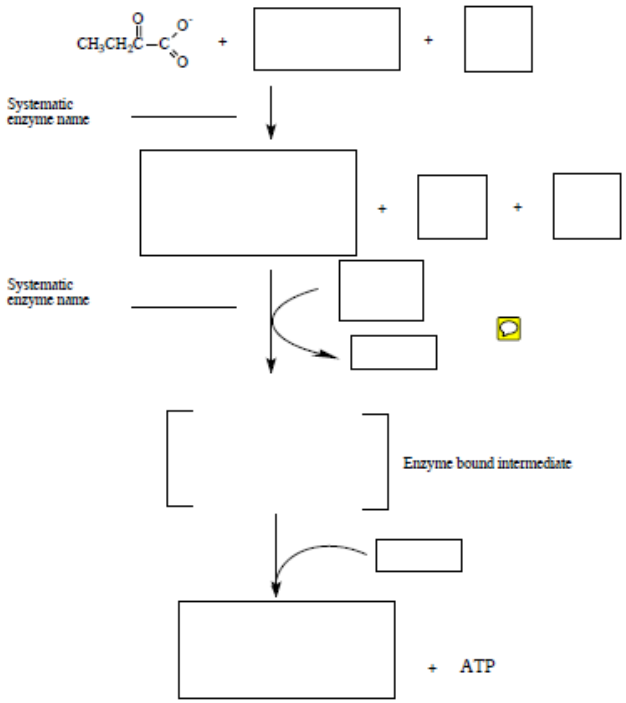
For example:

Positively Charged Species goes from **LOW** positively charged are to a **HIGH** positively charged area = unfavourable

Question 4

(a) (12 points) Complete the following bacterial SLP scheme adding structures and 1-word (semi) systematic enzyme names in the spaces provided. Abbreviations may be used for vitamin containing groups or molecules and for any molecules lacking a vitamin-derived group if the molecule consists of > 5 C atoms.

- Sometimes easier to work in the reverse direction
- Look for similarities with known reactions and enzyme
    - Has ATP as part of the reaction
    - Has an enzyme bound intermediate



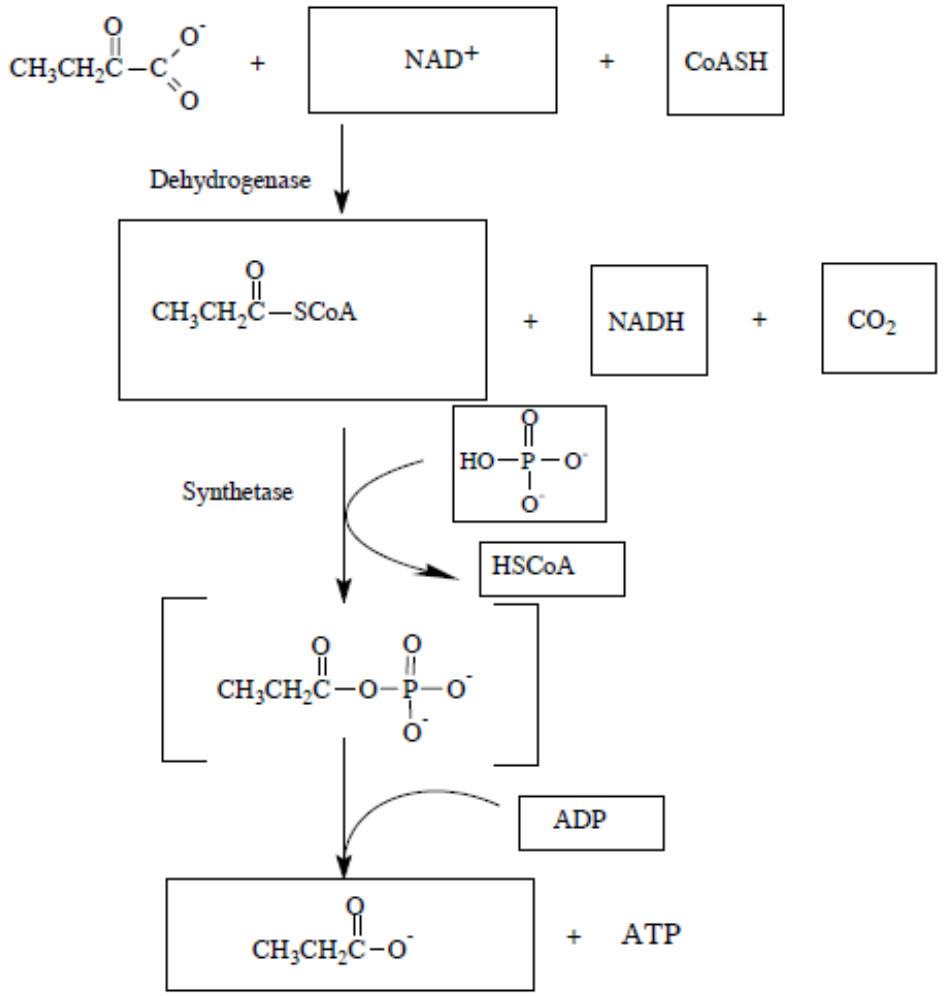
Similar to the usual synthetase enzyme reaction

Enzyme bound intermediate makes it similar to TCA 5

Thus, is CoA involved

First reaction could be similar to TCA 4

Question 4

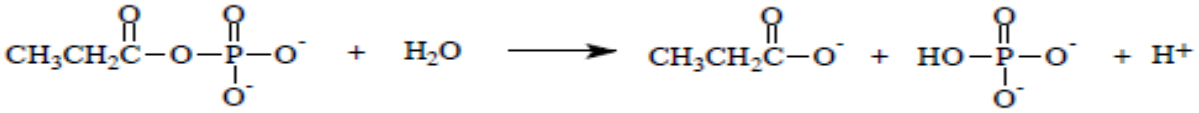


Question 4

(b) At some point in the reaction scheme from part (a) a high energy phosphorylated intermediate should appear.

(i) (2 points) Write the reaction for the imaginary hydrolysis of this molecule showing all structures.

- Look for high energy intermediate
- Write simple hydrolysis reaction equation



Note: Make sure all atoms are present on both sides (Be careful with protons

This is not an enzymatic reaction, thus protons should be conserved

Question 4

(ii) (4 points) The main reason that the hydrolysis reaction yields a large amount of energy is because bonding in the products is better stabilized by resonance than bonding in the reactants.

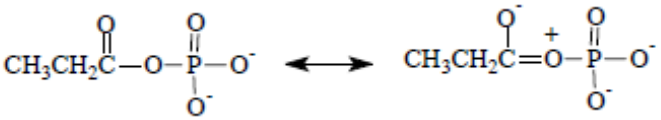
If you were going to illustrate this point, you would draw some RELEVANT resonance structures.

Draw two resonance structures for the reactants that you would use to illustrate why bonding in the products is better stabilized by resonance than bonding in the reactants.

Basically showing only half of a comparison between reactants and products

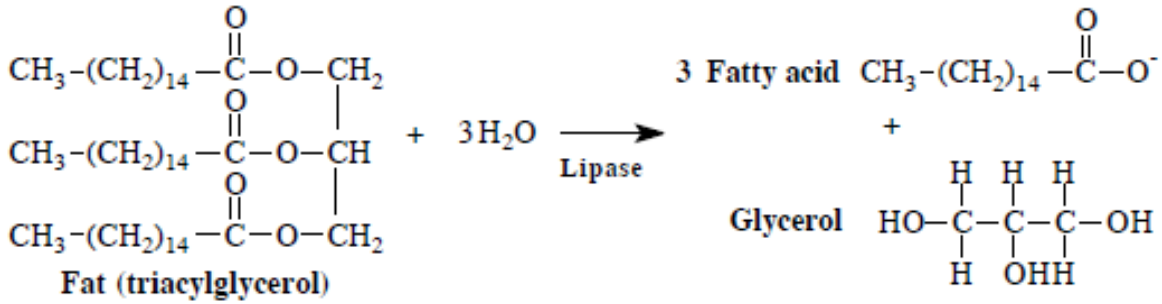
DO NOT show both reactants and products, in a full comparison

This is testing whether you know which resonance structures actually make a difference



Question 5

Triacylglycerols (fats) can be used to make ATP following their digestion by lipases which catalyze the hydrolysis of ester bonds lipids.



Fatty acids are linked to CoA and converted to Acetyl CoA. The following reaction sequence (called "-oxidation is used (Yes, you will not actually “learn” the pathway until BIOC302 but you have already learned enough to understand most of it).

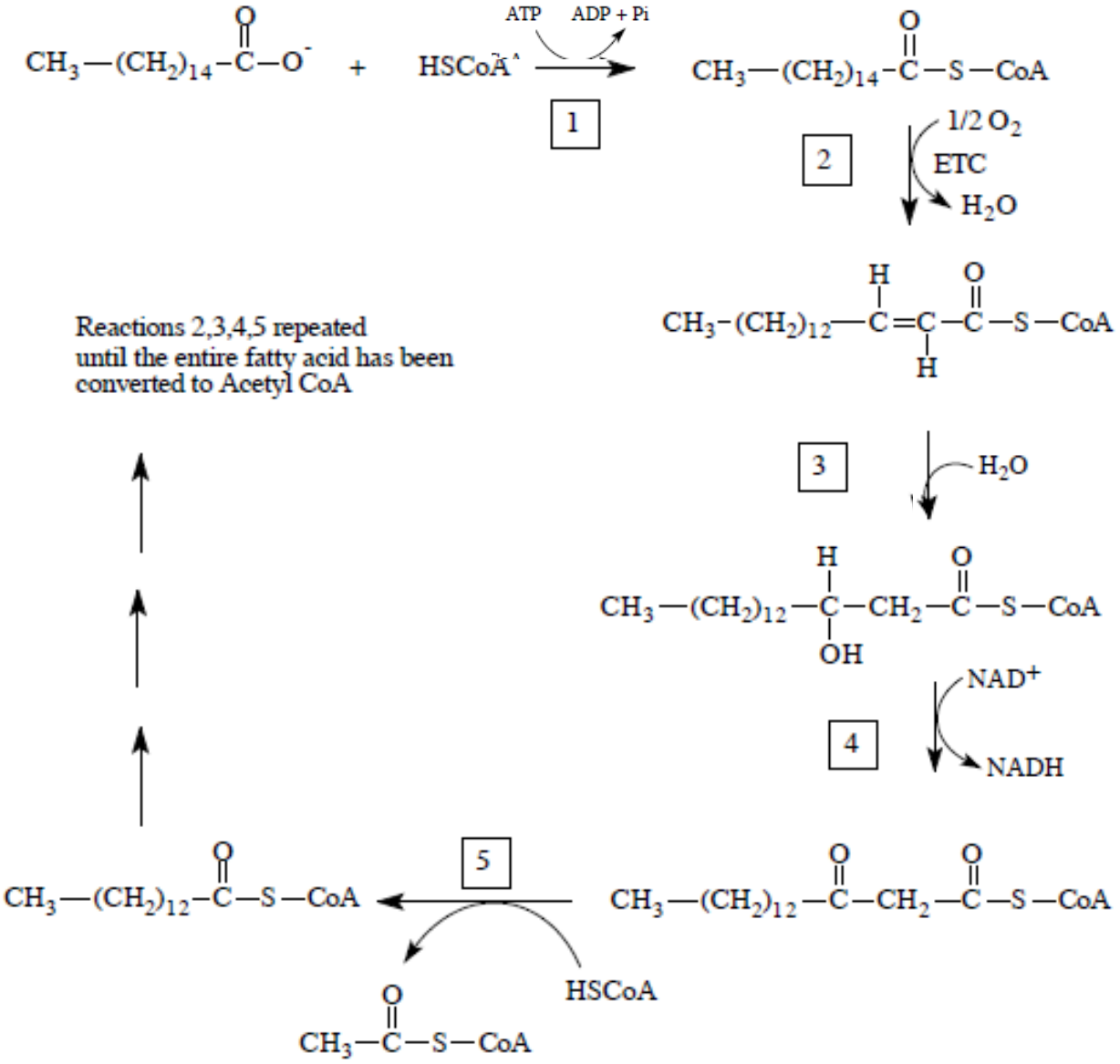
Question 5

(a) In the reaction sequence on p. 11 , some details have been omitted. Based on your knowledge of Glycolysis and the TCA cycle ADD any missing substrates, products and H+ required to balance reactions 1 to 4; no information is required about reaction 5. Abbreviations may be used for vitamin-containing groups or molecules and for any molecules lacking a vitamin-derived group if it has > 5 C atoms. (4 points)

Look for similarities between reactions in the pathway and reactions we have learnt in TCA cycle and Glycolysis

Look at differences between reactants and products

- Will tell you what has been added or taken away


ATP
ADP + Pi
HSCoA
1
2
1/2 O2
ETC
H2O
Reactions 2,3,4,5 repeated
until the entire fatty acid has been
converted to Acetyl CoA
3
H2O
4
NAD+
NADH
5
HSCoA

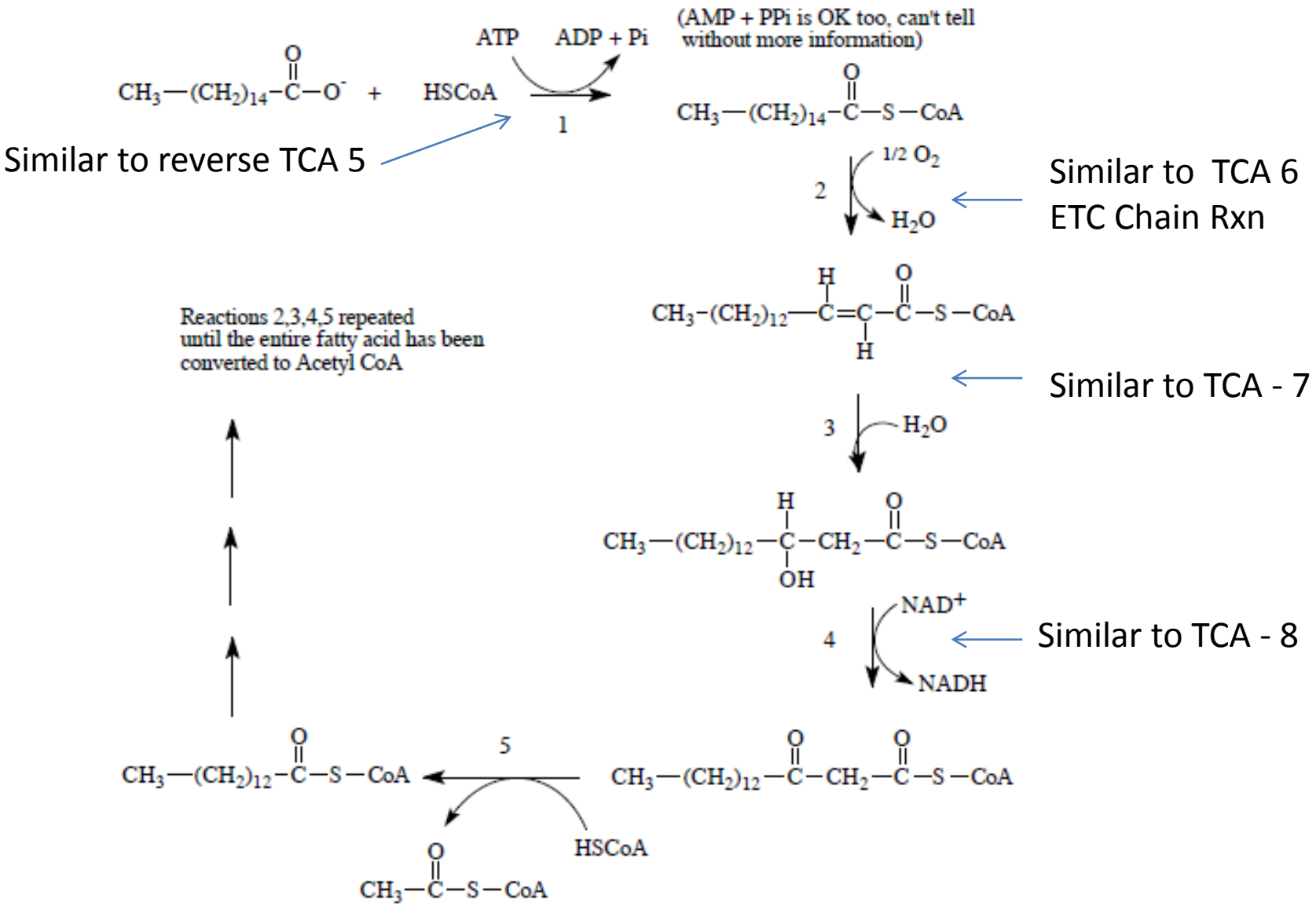
Question 5

(b) State the full name of the one vitamin that is involved in every reaction. (2 points)

Pantothenic acid

Recap:

Pantothenic Acid → CoA
Niacin → NAD+/NADH
Lipoic Acid → PDH
Thiamine → TPP
Riboflavin + Prosthetic Groups → Page 156


(AMP + PPi is OK too, can't tell without more information)
ATP
ADP + Pi
HSCoA
1
Similar to reverse TCA 5
2
1/2 O2
H2O
Similar to TCA 6
ETC Chain Rxn
Reactions 2,3,4,5 repeated until the entire fatty acid has been converted to Acetyl CoA
3
H2O
Similar to TCA - 7
4
NAD+
NADH
Similar to TCA - 8
5
HSCoA

Question 5c

Reaction	Reaction similar to which Glycolysis or TCA cycle reaction?	Systematic enzyme name (1 word)	Names of any vitamin(s) involved in reaction
1	TCA-5	Synthetase	None
2	TCA-6	Dehydrogenase	Riboflavin
3	TCA-7	Synthase or Lyase	None
4	TCA-8	Dehydrogenase	Niacin

Question 5

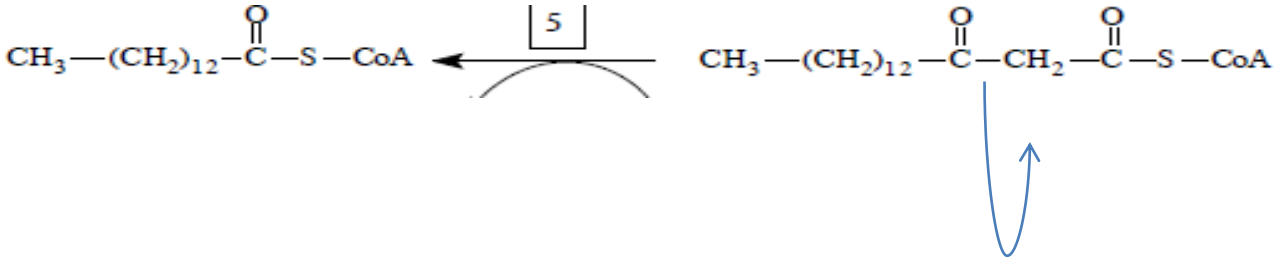
(d) In Rxn #5, a C-C bond is broken. How is the carbanion stabilized? It may be useful draw resonance structures here (4 points).

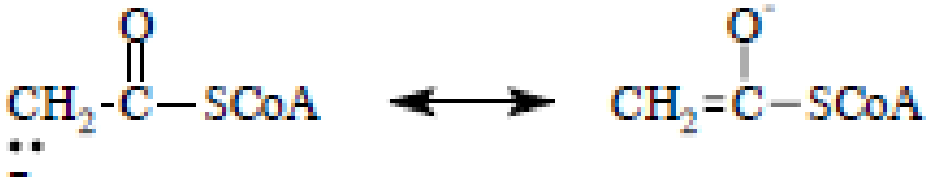
We know of two types of carbanion stabilization

Resonance stabilization w/ metal cation

Stabilization by TPP

Where is the C-C bond broken and where do the e- go?





Will use cation or positive residue to stabilize negative charge on O^-

Question 5

(e) Calculate the number of molecules of ATP formed when one 16-C fatty acid is converted to acetyl CoA and then oxidized completely to CO_2 and H_2O via the TCA cycle and the ETC. (6 points)

Be sure to include ATP from both SLP and oxphos and use the experimentally-derived values for ATP yields from oxidative phosphorylation.

Helpful Things to Remember (look over each cycle)

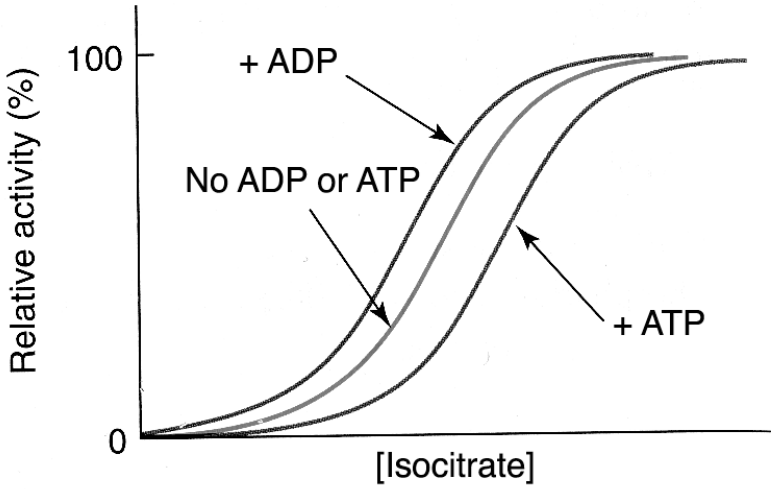
-TCA Cycle → 1 Cycle = 10 ATP
- 3 NADH
- 1 SLP = 1 ATP
- 1 ETC Donator (Succinate) = 1.5 ATP

-Glycolysis → 1 Glucose = Net 7 ATP
- 2 ATP Used
- 2 NADH
- 4 ATP made

Pyruvate → Acetyl CoA
- 1 NADH

Question 6

Isocitrate dehydrogenase (IDH) catalyzes one of the TCA cycle reactions. Kinetic studies of the enzyme as a function of isocitrate concentration ([NAD+] constant) gives the following results:



(a) (2 points) Is IDH an allosteric enzyme? How do you know? Yes

Talk about the curve of the graph

Talk about what happens to the graph when different substances are added

Question 6

(b) (2 points) Using the language of enzyme kinetics, what is ATP called? Be as specific as possible and explain briefly.

Negative Effector or Allosteric Inhibitor

Explain what happens to the geneal rxn rate in the presence of ATP

(c) (3 points) What is the biological significance of ATP's effect on IDH?

What does IDH make?

Why is this regulation needed for it?

What would happen if ATP regulation was not present?

Answer:

The IDH reaction yields NADH which is used to make ATP by oxidative phosphorylation. When the [ATP] is high, this indicates ATP exceeds the needs of the cell. By using ATP as an inhibitor of IDH unneeded NADH and thus unneeded ATP is not made.

Question 6

(b) (2 points) Using the language of enzyme kinetics, what is ADP called? Be as specific as possible and explain briefly.

Positive Effector or Allosteric Activator

Explain what happens to the general rxn rate in the presence of ADP

(c) (3 points) What is the biological significance of ADP's effect on IDH?

What does IDH make?

Why is this regulation needed for it?

What would happen if ADP regulation was not present?

Answer:

The IDH reaction yields NADH which is used to make ATP by oxidative phosphorylation. When the [ADP] is high, this indicates the cell's needs for ATP exceeds the supply. By using ADP as an activator of IDH moreNADH and thus more ATP is made to satisfy the cells needs for ATP..

Question 6

(f) (4 points) Based on the characteristics of the IDH reaction, would you expect the reaction to operate “close to equilibrium” or “far from equilibrium” in the mitochondrion. Explain briefly.

Far from equilbrium

WHY?

These enzymes usually catalyze near Vmax

Regulation keeps it from being active at low concentrations

Thus, Qcell builds up and is far from equilibrium

And when the enzyme is activated it has a lot of “Built Up” energy which allows it to work at Vmax (pg. 245)

Q & A

Rest of the session will be for any questions

... Otherwise GOODLUCK on the Final!