Name Key

Biochemistry 5700
Fall, 1999
Exam 3

INSTRUCTIONS:

Do not begin until 10:30 AM. The in-class portion of the exam must be turned in by 11:20 AM. The in-class portion should have 8 questions and 5 pages. **ANSWER ANY ~~7~~ 6 OF 8 OF THE QUESTIONS- Mark "DO NOT GRADE" on the one question you choose to skip. DO NOT answer all eight questions-if you do, cross the one out which you don't want graded.** Each graded question is worth 10 points. The take home portion contains two questions, worth a total of 30 points. The take home portion is due beginning of class Wednesday. Good Luck!!

6 8 8

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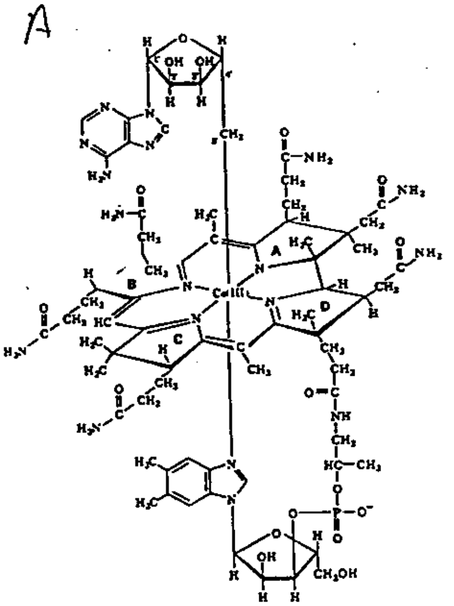
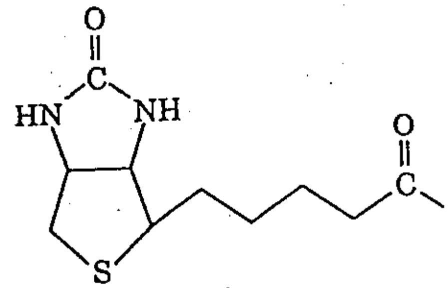
(1) Provide the names for the following cofactors, and name an enzyme that uses that cofactor.

A



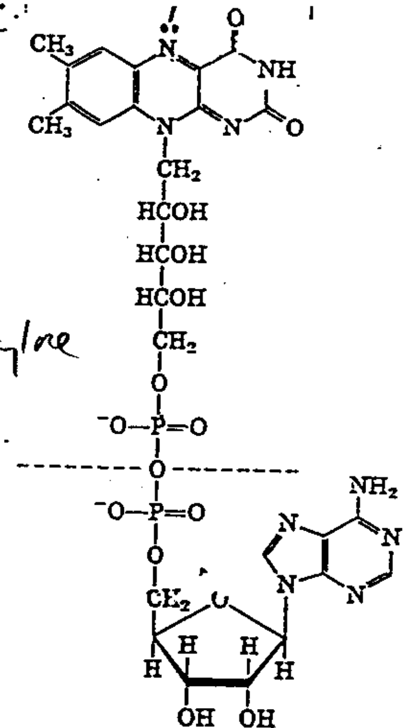
deoxyadenosylcobalamin
methylmalonyl CoA mutase
(succinyl CoA synthase?)

B



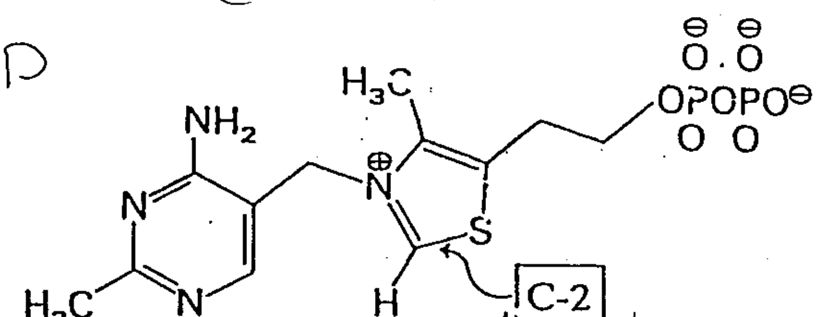
biotin
propionyl-CoA carboxylase

C



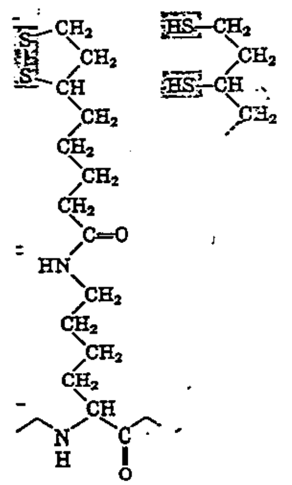
Flavin adenine dinucleotide
PDH complex

D



thiamine pyrophosphate
pyruvate decarboxylase

E



lipoamide, ox red.
PDH complex

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(2) You have just eaten a big juicy, fatty hamburger. Explain how the portion of the hamburger that is fat eventually arrives in your muscle cell (i.e., what is the biochemical/molecular path from intestine to muscle?

The fat in the small intestine is emulsified by bile salts, forming soluble mixed micelles. The fat is then hydrolyzed by lipases to form free FA + glycerol. The free acids enter intestinal mucosa, where they are reesterified to TAGs, then packaged with cholesterol + proteins into lipoprotein particles called chylomicrons. The chylomicrons enter the lymph + blood vessels, + are transported to tissues. Lipoprotein lipases cleave the chylomicrons in the capillaries, releasing glycerol + the FAs. The fatty acid then enter muscle cells.

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(3) For the following general classes of enzymes, name a specific enzyme of that class, and write the balanced reaction catalyzed by the enzyme (note-structures and mechanism are not required-just names).
(a) claisen condensation

Citrate synthase : oxaloacetate + acetyl-CoA → citrate + HSCoA

(b) elimination
enolase: 2-phosphoglycerate → H_2O + phosphoenolpyru

(c) phosphoryl transfer: mutase
phosphoglycerate mutase: 3-phosphoglycerate → 2-phospho glycer

(d) isomerization
phosphoglucoisomerase: glc-6-P → Frc-6-P

(e) thiolysis
β-Ketoacyl-CoA thiolase: 2-ketoacyl-CoA + HSCoA → Acetyl-CoA + $R-\overset{O}{\overset{\|}{C}}-SCoA$ (Fatty acyl-CoA)

(4) The K_m for glucose of glucokinase (liver) is 10 mM while that of hexokinase (muscle) is 0.1 mM. Explain.

The muscle hexokinase has a higher affinity for glucose (ie, lower Km), allowing conversion of glc → glc-6-P under all phys. concentrations of available glucose. This is a reflection of the need for muscle to continually oxidize glucose for conversion to mechanical energy. The liver, in contrast, does not perform mechanical work; in contrast, its role is regulating glucose flux. Here, at low blood glucose concentrations glucokinase will not convert glc → glc-6-P, but will instead export glucose via synthesis and/or glycogen breakdown. Only when blood [glc] is very high will glucokinase operate & commit glucose to glycolysis, glycogen synthesis, & ox. pentose pathway in the liver.

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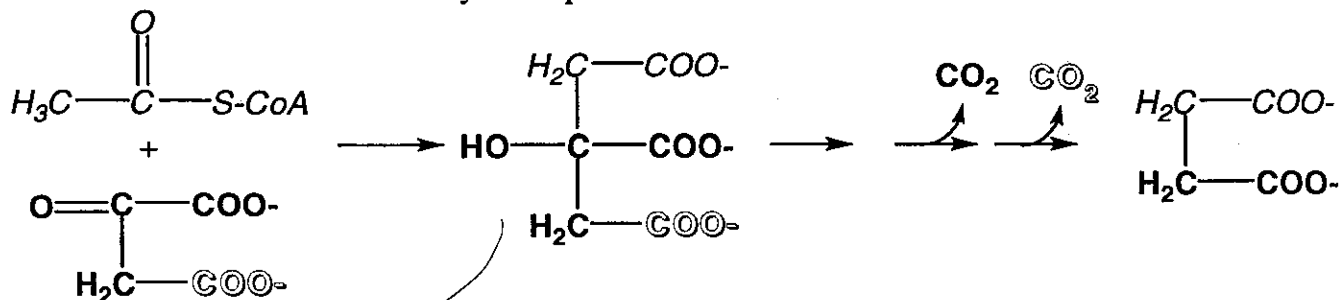
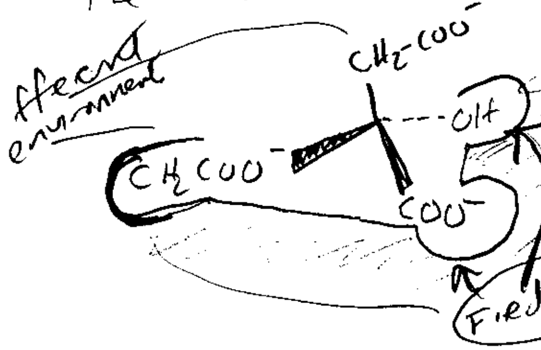
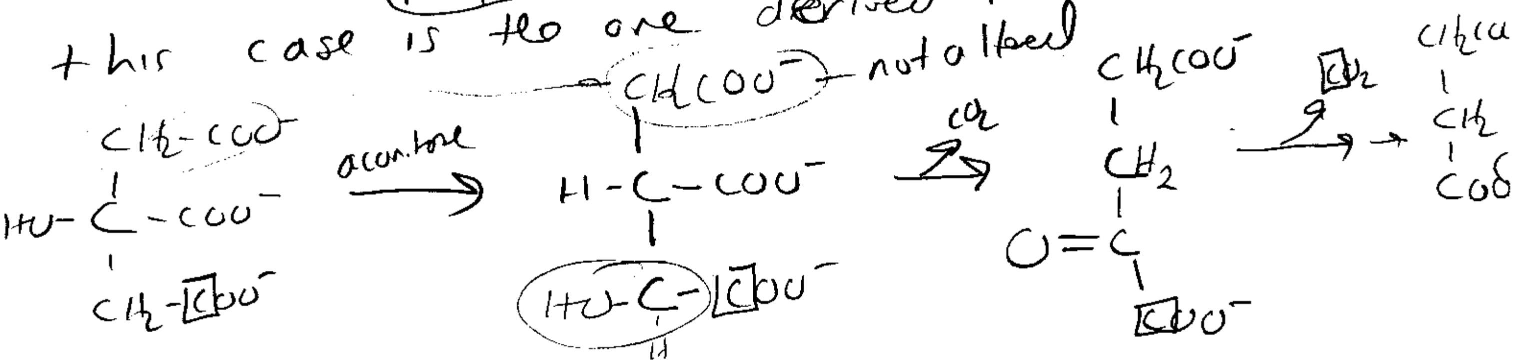
(5) The following figure shows the fates of carbon atoms undergoing conversion from OAA and acetyl-CoA to succinate in the citric acid cycle. Explain the molecular basis for these results.



Although citrate is not a chiral molecule, it can behave as such if an enzyme binds it in such a way that the 2 (CH_2-COO^-) arms must necessarily occupy different spatial areas. Such is the case for aconitase, as illustrated below:



This stereospecificity of binding requires that only 1 of the 2 CH_2COO^- undergoes reaction so the -OH is all transferred to the reactive CH_2COO^-, which in this case is the one derived from OAA:



Discrimination of "prochiral" groups is an important feature of the specificity of enzymes

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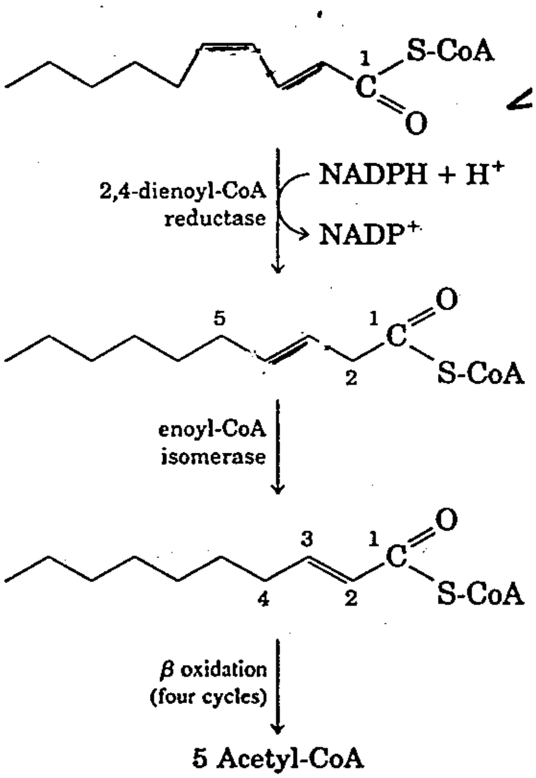
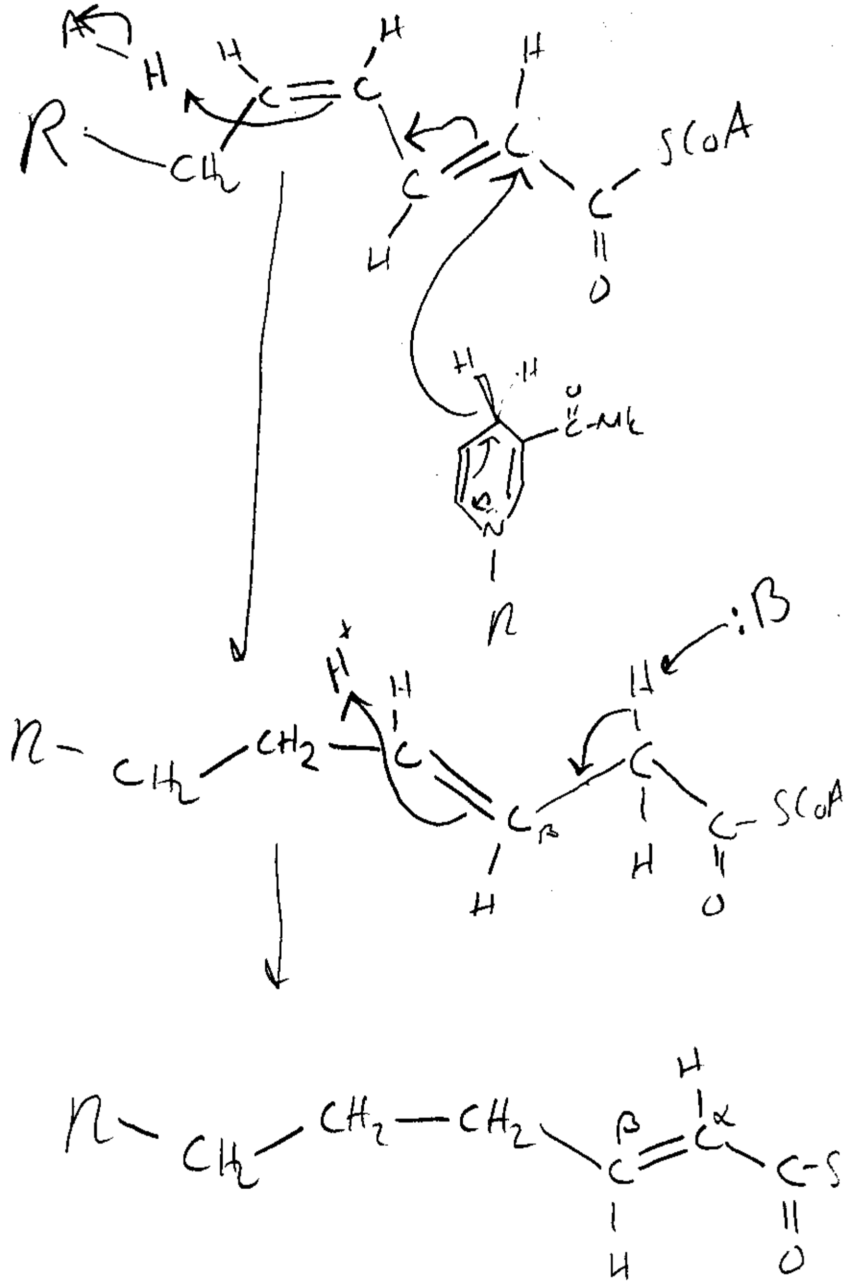
6) Write a complete reaction mechanism for the following two transformations:





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(7) Glycogen phosphorylase in the muscle is subject to positive/negative (circle the correct one) allosteric regulation by AMP while glycogen phosphorylase in the liver is subject to positive/negative (circle the correct one) allosteric regulation by glucose. Explain these differences.

In the muscle, you want to mobilize glycogen → glucose in situations where E is needed. A signal of low E status is the presence of AMP. Since covalent regulation of glycogen phosphorylase (on/off, responding to hormones) is relatively slow, the muscle cell can still partially activate glycogen phosphorylase in emergency situation (eg fight or flight) in response to low AMP, buying time for the full-blown hormonal activation.

A function of the liver is to regulate glucose metabolism in other tissue. Thus, if glycogen phosphorylase is in the phosphorylated active form, it will convert glycogen → glucose for export. If blood glucose suddenly rises due to a dietary source, the liver will now want to ~~store~~ glc as glycogen rather than break it down. The dephosphorylation of glycogen phosphorylase takes time. A quick fix is to slow the "active" enzyme down by glucose allosterically.

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(8) Short answers. Answer each question in one sentence in the space provided.

(a) What are the two purposes of the oxidative portion of the pentose phosphate pathway?

Make NADPH & ribose

(b) What is substrate level phosphorylation?

synthesis of ATP by phosphoryl transfer from an organic donor molecule.

(c) What is the purpose of performing lactic acid fermentation in the muscle?

regenerate oxidized form of NAD (NAD^+) to sustain further glycolysis when O_2 is limiting

(d) Why does the conversion of acetyl-CoA to CO2 ocur by a cyclic rather than linear pathway?

C4 & C5 & C6 cmpds in cyclic pathway can be shunted off for other biosynthetic/catabolic uses.

(e) Why can't we live solely on fat as a source of carbon?

C2 units, in mammals, cannot be used for net synthesis of C_3 & longer cmpds needed to replace citric acid cycle intermediates & to meet other biosynthetic needs

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Take home portion of Exam 3: You may consult any reference texts you like when working this portion. You are to work the problems individually- do not discuss with fellow students, faculty, etc. Turn in your solutions to me at beginning of class **Wednesday Dec 1.**

For thermodynamic and electrochemical data and constants, refer to your text, inside cover (for conversions) and chapter 13.

(1) (20 points) The hydrolysis of lactose to D-galactose and D-glucose occurs with a $\Delta G°'$ of -4.0 kcal/mol.

7 (a) Calculate K_{eq}' for this hydrolysis.

6 (b) What are $\Delta G°'$ and K_{eq}' for synthesis of lactose from D-galactose and D-glucose?

7 (c) Lactose is synthesized in the cell from UDP-galactose and D-glucose in a reaction catalyzed by lactose synthase. Given that $\Delta G°'$ for hydrolysis of UDP-galactose is -7.3 kcal/mol, calculate $\Delta G°'$ and K_{eq}' for the reaction catalyzed by lactose synthase.

(2) (10 points) You have a solution of cytochrome c poised at a potential of 0.30 V and pH 7.0. What percentage of the cytochrome c is in the oxidized state?

1 cal = 4.184 J

note!

R = 8.314 J/mol K

or

1.987 cal/mol K

Key, Take Home portion, Exam 3

1) lactose + $H_2O \rightleftharpoons$ D-gal + D-glc

a) $\Delta G^{\circ\prime} = -RT \ln K_{eq}'$; $K_{eq}' = e^{-\Delta G^{\circ\prime}/RT}$

$$K_{eq}' = e^{(4\,\cancel{kcal/mol})/(1.987\,\cancel{cal/mol\,K})\left(\frac{1000\,cal}{kcal}\right)\left(\frac{1}{298\,K}\right)}$$

$\boxed{K_{eq}' = 859}$ (660 at 310 K)

b) D-gal + D-glc $\rightleftharpoons$ lactose + H_2O

$\boxed{\Delta G^{\circ\prime} = +4.0\ kcal/mol}$ (16.74 kJ/mol)

$\boxed{K_{eq}' = 1/859 = 0.0012}$

c) UDP-gal + D-glc $\rightarrow$ UDP + lactose

UDP-gal + $\cancel{H_2O}$ $\rightarrow$ $\cancel{\text{D-gal}}$ + UDP $\Delta G^{\circ\prime} = -7.3$
$\cancel{\text{D-gal}}$ + D-glc $\rightarrow$ lactose + $\cancel{H_2O}$ $\Delta G^{\circ\prime} = 4$

$\Delta G^{\circ\prime} = -3.3$

$\boxed{\Delta G^{\circ\prime} = -3.3\ kcal/mol}$ = (−13.8 kJ/mol)

$$K_{eq}' = e^{-(-3.3\,\cancel{kcal/mol})\left(\frac{1}{1.987\,\cancel{cal/mol\,K}}\right)\left(\frac{1000\,cal}{kcal}\right)\left(\frac{1}{298}\right)}$$

$\boxed{K_{eq}' = 263}$

$$CytC_{ox} + e^- \rightleftharpoons CytC_{red}$$

(2) $$\mathcal{E}' = \mathcal{E}^{\circ\prime} - \frac{RT}{nF} \ln \frac{cytC_{red}}{CytC_{ox}}$$

$$\frac{RT}{nF} \ln \frac{cytC_{red}}{CytC_{ox}} = \mathcal{E}^{\circ\prime} - \mathcal{E}'$$

$$\ln \frac{cytC_{red}}{CytC_{ox}} = \frac{(\mathcal{E}^{\circ\prime} - \mathcal{E}')\,nF}{RT}$$

$$\frac{CytC_{red}}{CytC_{ox}} = \exp\left[(\mathcal{E}^{\circ\prime} - \mathcal{E}')\left(\frac{nF}{RT}\right)\right.$$

$$\frac{cytC_{red}}{cytC_{ox}} = \exp \frac{(0.235 - 0.30\,\cancel{V})(1)(96{,}485\,\frac{J}{\cancel{V\,mol}})}{(8.3144\,J/\cancel{mol\,K})(298\,\cancel{K})}$$

$$\frac{CytC_{red}}{CytC_{ox}} = 0.0796$$

$$CytC_{red} = 0.0796\, cytC_{ox}$$

$$CytC_{red} + CytC_{ox} = 1$$

$$CytC_{red} = 1 - CytC_{ox}$$

$$(1 - CytC_{ox}) = 0.0796\, cytC_{ox}$$

$$1.0796\, CytC_{ox} = 1$$

$$CytC_{ox} = 92.6\%$$

7.4% red