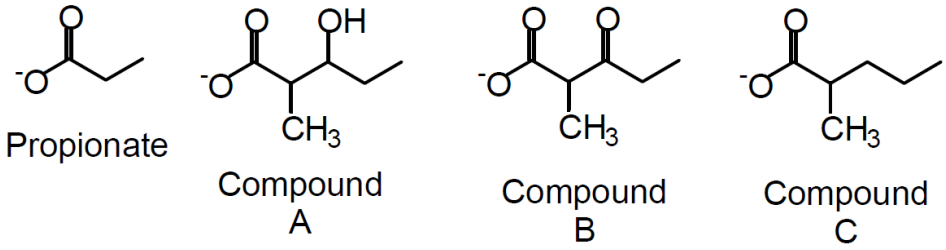
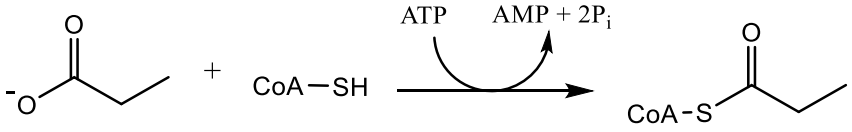
**Chemistry 5.07 2013**
**Problem Set 10 Answers**

## Problem 1

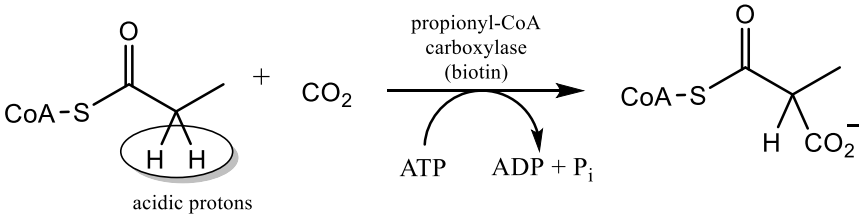
Many of our antibiotics are produced from soil microorganisms by a pathway called polyketide biosynthesis. A bacterium was isolated from the Thai rainforest that produces Compounds A, B and C, which are the precursors to important antibiotics. [This pathway is similar to one described on a PowerPoint presentation given in class.] The bacterium uses propionyl CoA (derived from propionate) instead of acetyl CoA. Instead of acetyl CoA carboxylase (which makes malonyl CoA), it uses propionyl CoA carboxylase to start its fatty acid biosynthesis pathway. Based upon what you know of the general principles of fatty acid biosynthesis, please show how compounds A, B and C are made. Start your synthesis with the formation of the appropriate –CoA ester. If cofactors are involved, please indicate which ones.



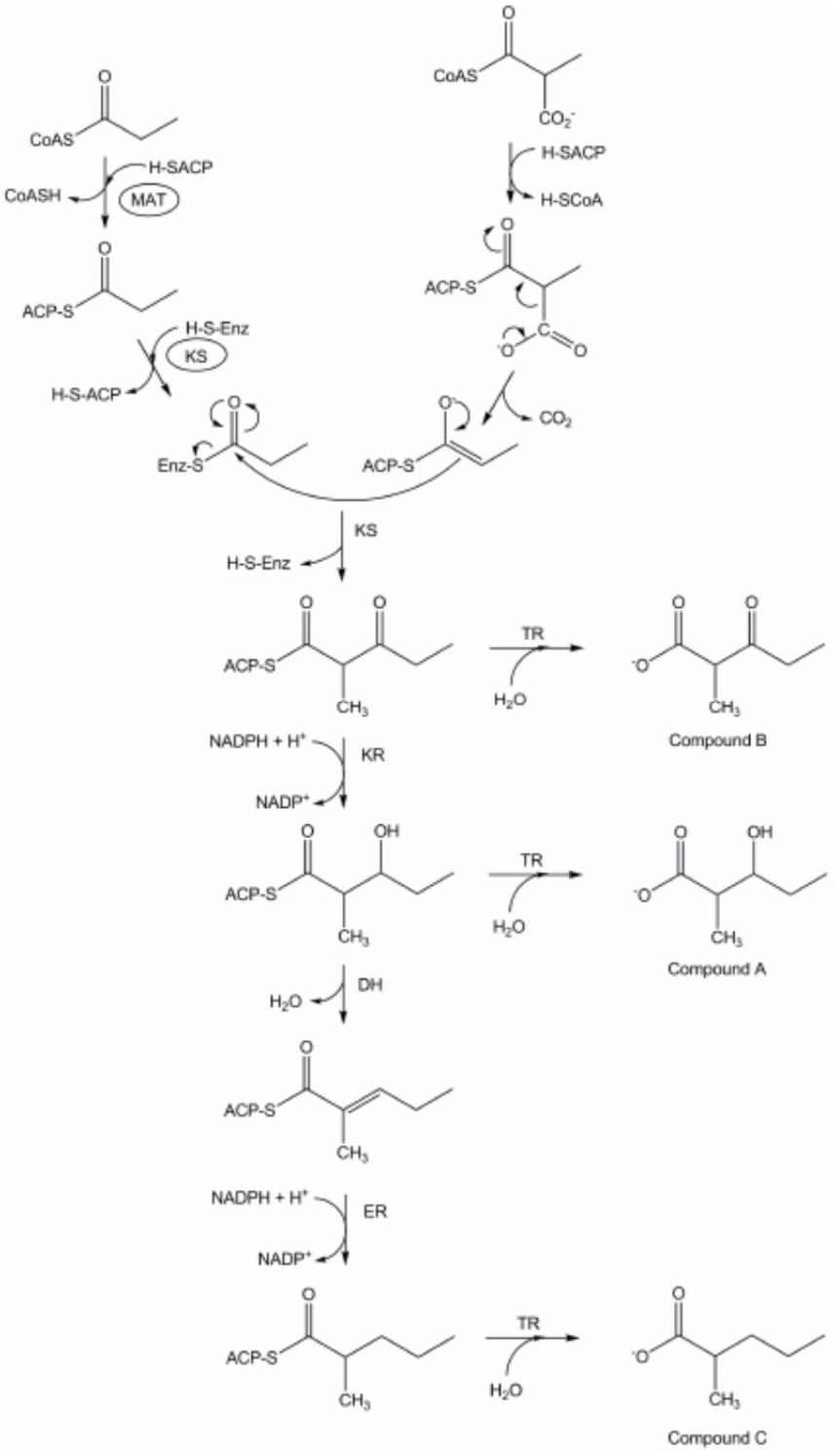

**Answer:**
**First, propionic acid will be converted into propionyl-CoA via an acyl-CoA synthetase.**




**One equivalent of propionyl-CoA will then be carboxylated via a biotin-dependent mechanism, similar to that of acetyl-CoA carboxylase.**




**The product, methylmalonyl-CoA, can then undergo a condensation with a second equivalent of propionyl-CoA as shown below. At three different points in this reaction, the intermediate can leave the reaction and the thioester bond can be hydrolyzed to yield the fatty acid (compounds A, B and C). At each step, the relevant activity of the FAS is indicated.**


CoAS
CoASH
H-SACP
MAT
ACP-S
H-S-Enz
KS
H-S-ACP
Enz-S
CO2
H-SCoA
KS
H-S-Enz
TR
H2O
Compound B
NADPH + H+
NADP+
KR
OH
Compound A
DH
ER
Compound C

## Problem 2

A well fed rat is given an injection of $^{14}$C-acetate with the radiolabel in the -$CH_3$ group. Two hours later, the rat is sacrificed and triacylglycerides (TAGs) and glycogen are analyzed for the distribution of radioactivity in each.

**a.** Would you expect the levels of radioactivity in glycogen and TAGs to be similar or different? If different, which would have more? Explain.

**b.** Draw the structure of a TAG and show which carbon(s) would be most heavily radiolabeled.

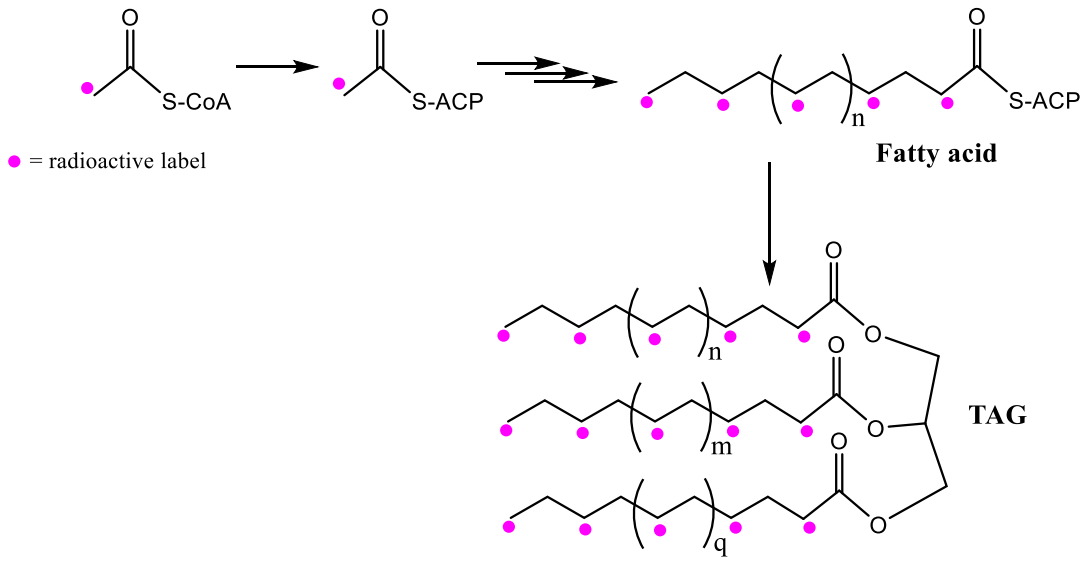
**c.** You repeat the experiment with another well fed rat, but this time you inject $^{14}$C-labeled glutamate with the label on the β-carbon. Show the label distribution in TAGs and glycogen and justify your answer.

**ANSWER:**

**a. Acetate is converted into acetylCoA, by using ATP to activate the carboxylate (adenylation) and forming a phosphoanhydride that can react with HSCoA.**

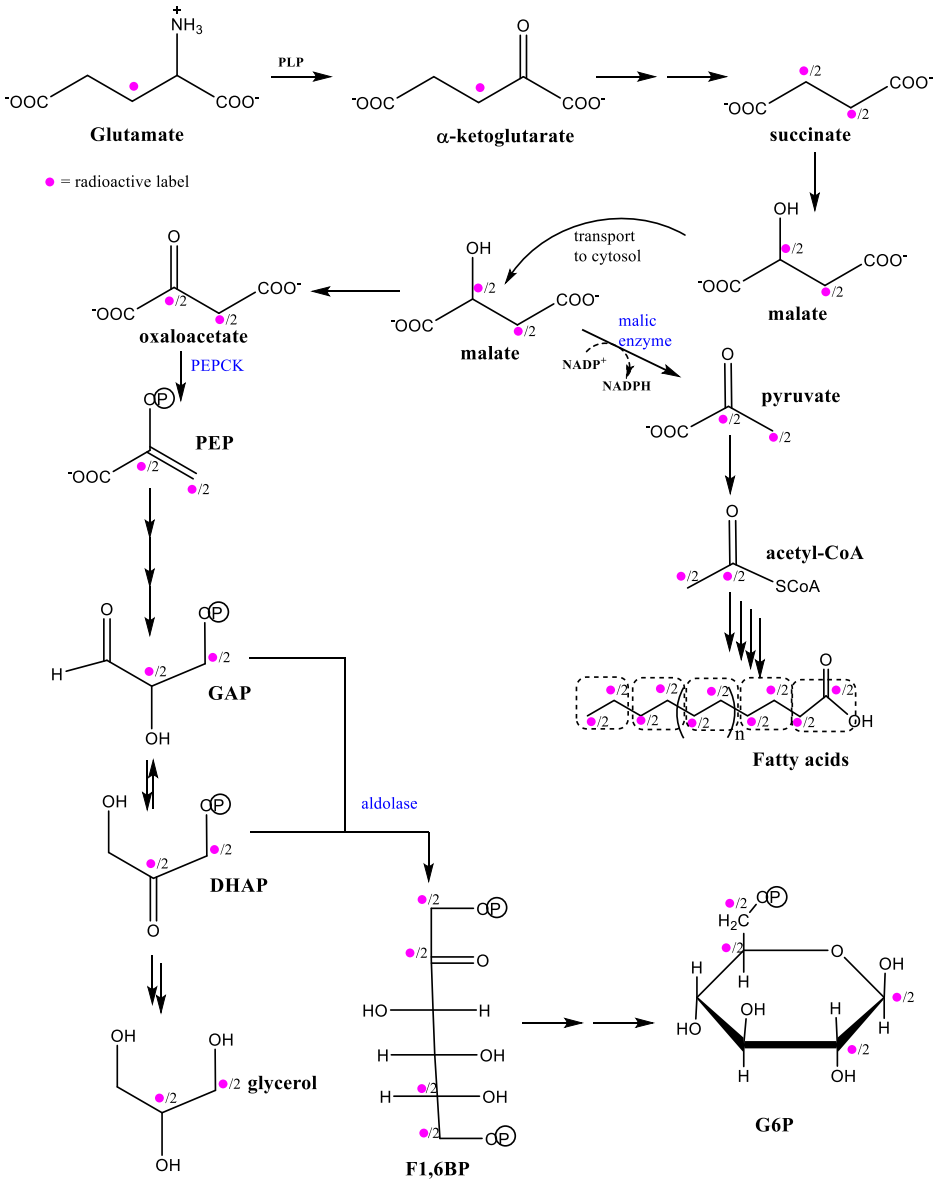
**The question here is to figure out which molecules can be made using acetylCoA. As you have learned, acetylCoA is a ketogenic precursor – it can form ketone bodies, fatty acids, steroids, etc. It cannot, however, be converted into glucose. Therefore, we expect very low levels of radioactivity in glycogen, but significant levels in TAGs. Within a TAG, we expect radioactivity only in the fatty acids; glycerol cannot be made from acetylCoA.**

**b. The label will be on the even carbons of the fatty acids.**



**c. Glutamate is a gluconeogenic source, so it will label both glucose (glycogen) and TAGs (on both fatty acids and glycerol).**

**By generating succinate, the label scrambles early on, so while each PEP or acetylCoA will have exactly <u>one</u> labeled atom, the probability is 50% that it would be one or the other. Therefore, the fatty acids generated from this acetylCoA pool will have a labeled atom in every group of 2 (ie. a (16,0) fatty acid will have exactly 8 labeled carbons, 1 in every 2 carbon fragments). Following PEP through gluconeogenesis, we find that glucose will have 2 labeled carbons, one being either C1 or C2, the other being either C5 or C6. Similarly, glycerol will have exactly one label, on either C1 or C2.**



## Problem 3

Gluconeogenesis involves the synthesis of glucose from non-carbohydrate precursors. The organs most active from the perspective of gluconeogenesis are the liver and the kidney, which supply glucose to the organs that cannot synthesize it, yet have a strict need for glucose as an energy source.

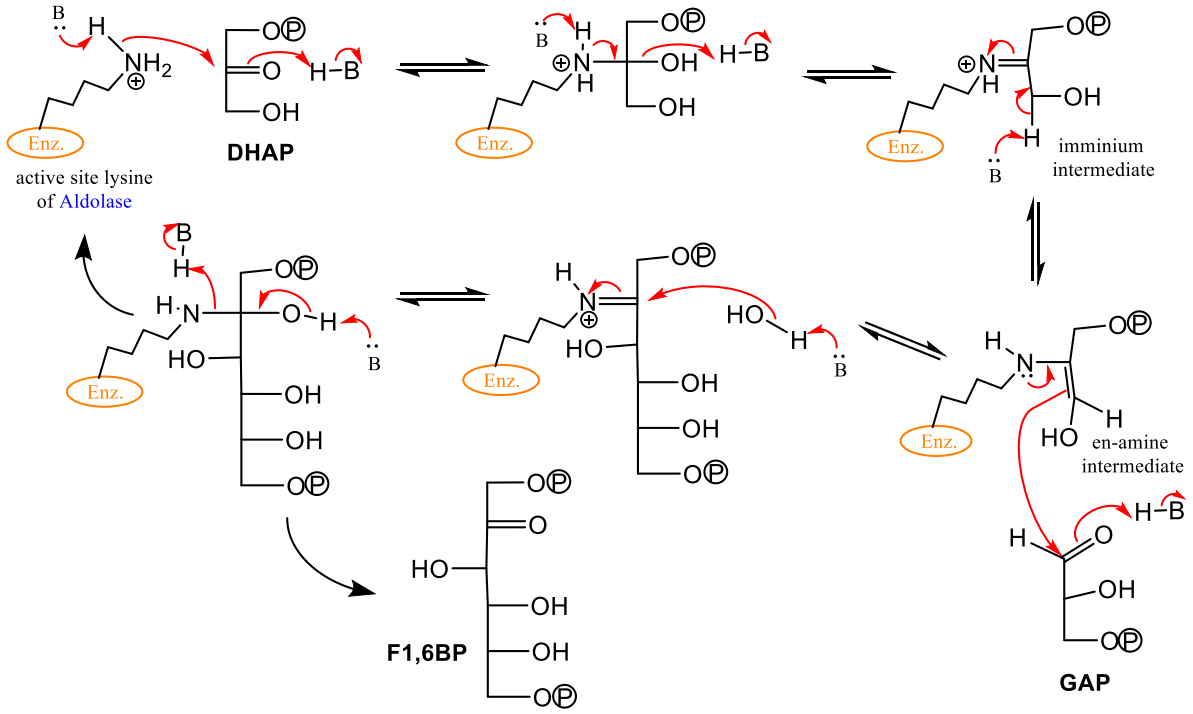
**a.** Gluconeogenesis requires several equilibrium steps of glycolysis to run in the reverse direction. Write out in detail the mechanism of the conversion of GAP and DHAP to F1,6BP. Is this reaction under standard conditions spontaneous in the direction of gluconeogenesis? The table on page 511 of the book may be helpful.

**b.** Similarly, write out the detailed mechanism of how GAPDH catalyzes the conversion of 1,3BPG to GAP. Show how its cofactor participates in the reaction. Is this reaction spontaneous under standard conditions? Page 499 in the book may contain some helpful information.

**c.** Glycolysis as a ten-step pathway from glucose to pyruvate is spontaneous. Is gluconeogenesis spontaneous under standard conditions (ie. is the $\Delta G^{o'}$ for the pathway negative)?

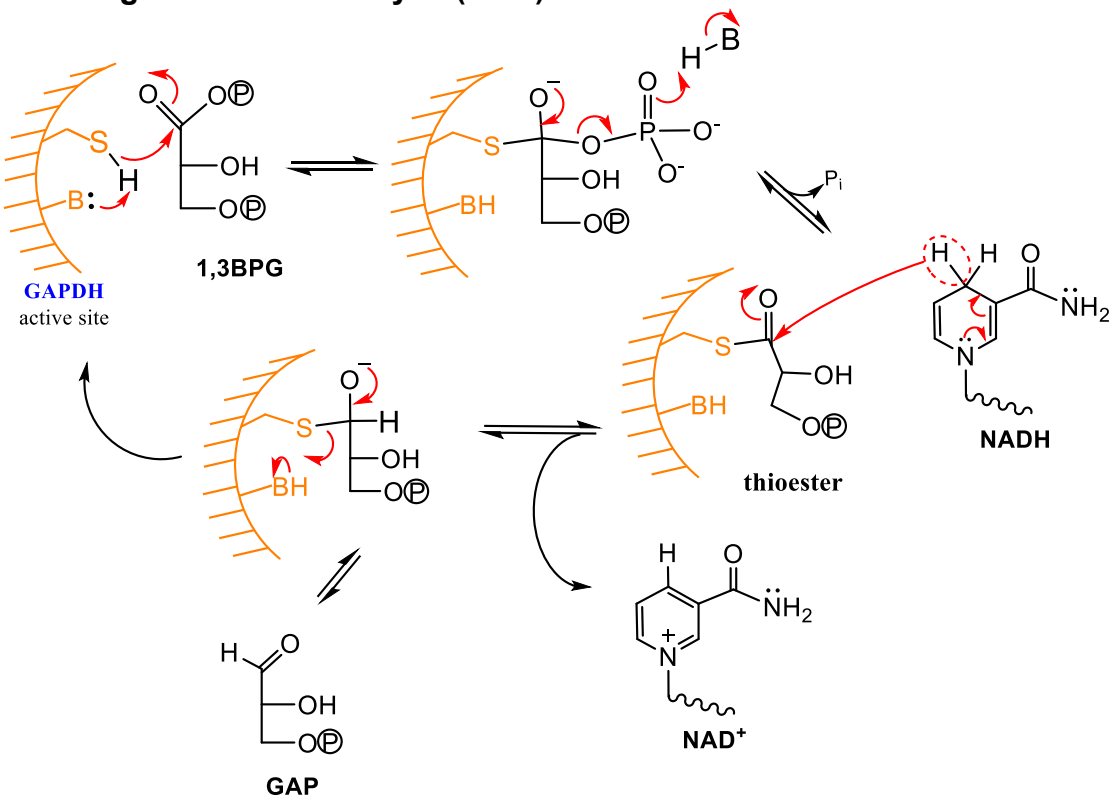
**ANSWER:**

**a. The mechanism is a direct aldol reaction. The active site lysine of the aldolase forms an iminium ion with DHAP, favoring the formation of the reactive en-amine. This reacts with the aldehyde group of GAP to give the 6 carbon frame of F1,6BP.**



**The $\Delta G^{o'}$ of the glycolysis aldolase reaction is +22 kJ/mol. (according to the table in the book, Voet & Voet 3rd ed. page 511). This means the formation of F1,6BP is in fact favorable, $\Delta G^{o'}$ = -22kJ/mol ). Of course, in living cells, the actual $\Delta G$ will depend on the concentrations of the reactants, which will determine which pathway (glycolysis or gluconeogenesis) is favored.**

**b. Similar to the direct GAPDH reaction, this reaction involves formation of a thioester with the active site cysteine on GAPDH. The thioester is then reduced by NADH to generate an aldehyde (GAP).**




**As you recall from glycolysis, the GAPDH reaction has a slightly positive $\Delta G^{o'}$ in the forward direction. Therefore, the reverse reaction has a negative $\Delta G^{o'}$ and it is thus favored. Again, just like the aldolase step, the concentrations of the reagents play a very big role in determining in which direction the reaction will proceed.**

**c. Gluconeogenesis as a pathway is also spontaneous. Note however, that unlike glycolysis, gluconeogenesis from pyruvate to glucose requires net consumption of ATP (i.e., the pyruvate carboxylase step, PEP-CK step and the phosphoglycerate kinase step). It is the energy of all these ATPs that ultimately allows the pathway to be spontaneous.**

# 5.07SC Biological Chemistry I
Fall 2013