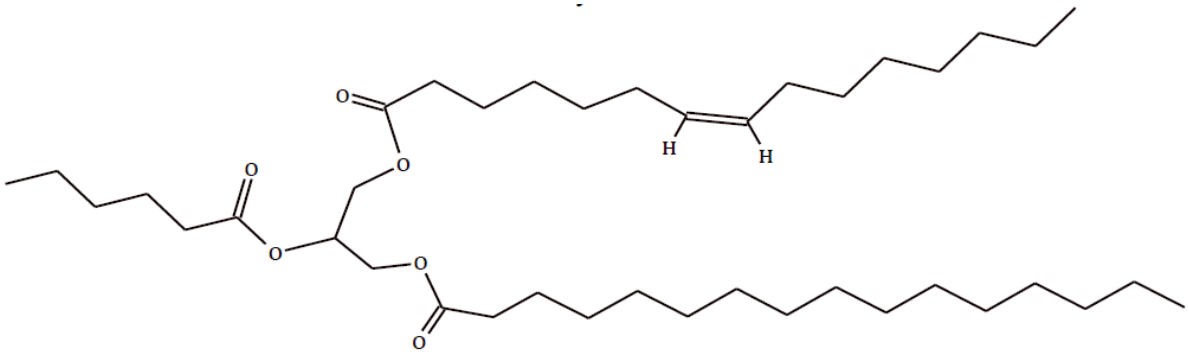
Chemistry 5.07/ BE 20.507
Answers to Problem Set 9 2013

Problem 1

The triacylglycerol drawn below acts as an energy source when administered to an organism from its diet. You may assume that it is delivered by chylomicrons to the target cell intact and that lipoprotein lipase fully hydrolyses the compound to its constituents, which are absorbed into the



cell efficiently.

a. Showing your reasoning, calculate the maximum amount of ATP that could be generated by the full oxidation of the compound.

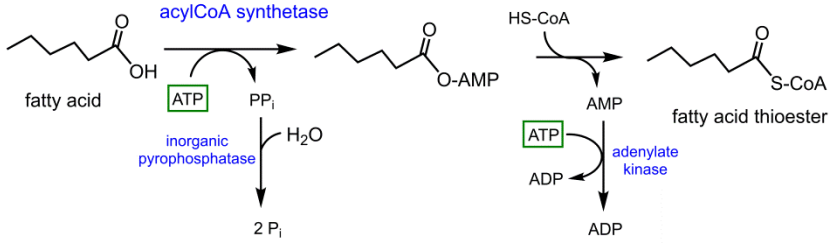
b. One of the arms has the same number of carbons as glucose. Compare the maximum yield of ATP achievable from full oxidation of that fatty acid, as compared to the ATP yield from glucose.

Answer:

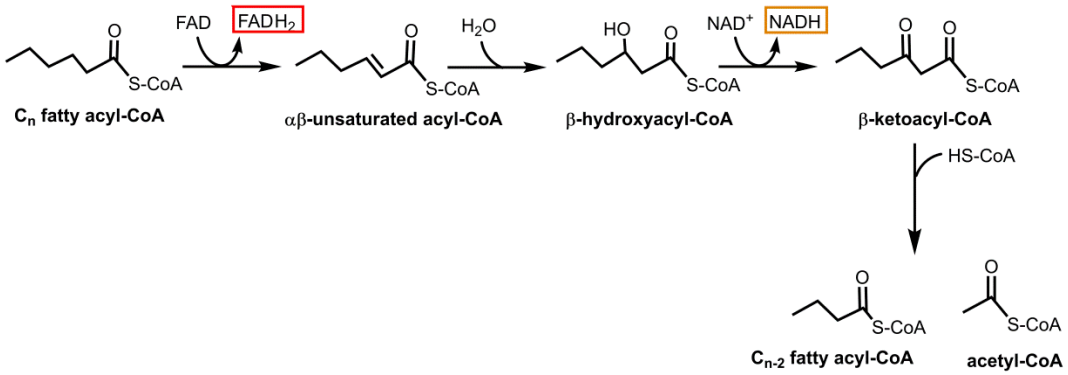
a. For the triacylglycerol (TAG) to be fully oxidized, it must be broken down into its components, i.e: glycerol and fatty acids. This step is accomplished using a lipase that hydrolyzes the ester bond using the catalytic triad that was also used by serine proteases. Before the fatty acids are oxidized, they are first activated through formation of an acyl-CoA species. Formation of the thioester bond primes the fatty acid for complete oxidation through β oxidation. The acylation reaction is catalyzed by acyl-CoA synthetases using ATP (converted to AMP and pyrophosphate (PPi)).

$$\boldsymbol{Fatty\ acid + CoA + ATP \rightleftarrows acyl\text{-}CoA + AMP + PP_i}$$

The breakdown of the pyrophosphate into inorganic phosphate (Pi), catalyzed by the inorganic pyrophosphatase enzyme helps drive the equilibrium of this process to the right. To recycle the AMP, a second equivalent of ATP is needed to make ADP, which is the substrate used by the complex V of mitochondria to regenerate ATP. Hence, the overall reaction of fatty acid activation requires 2 equivalents of ATP. The detailed reaction scheme for a C6 fatty acid is:



β oxidation has 4 steps, each catalyzed by a different enzyme, as shown below.



The first three steps are similar to the $\boldsymbol{succinate \longrightarrow oxaloacetate}$ reactions of TCA. The single bond between the α and β carbons is oxidized to a double bond using FAD and the enzyme acyl-CoA dehydrogenase. The $FADH_2$ generated from this reaction can enter the electron transport chain at the membrane bound electron transfer protein (ETF). The double bond is hydrated using enoyl-CoA hydratase, and the alcohol functionality is further oxidized to a ketone using an NAD^+-dependent mechanism. The β-ketoacyl-CoA then undergoes cleavage of the $C_\alpha - C_\beta$ bond to generate an acetyl-CoA and an acyl-CoA. This entire process will yield one equivalent of $FADH_2$ and one equivalent of NADH, thus producing 5 ATPs. The acetyl-CoA can enter TCA where it will generate a total of 12 more ATPs. Ultimately, the

entire fatty acid will be split into 2-carbon units (acetyl groups) that can be fully oxidized through TCA. For odd-chain and unsaturated fatty acids, this pathway is altered (see below).

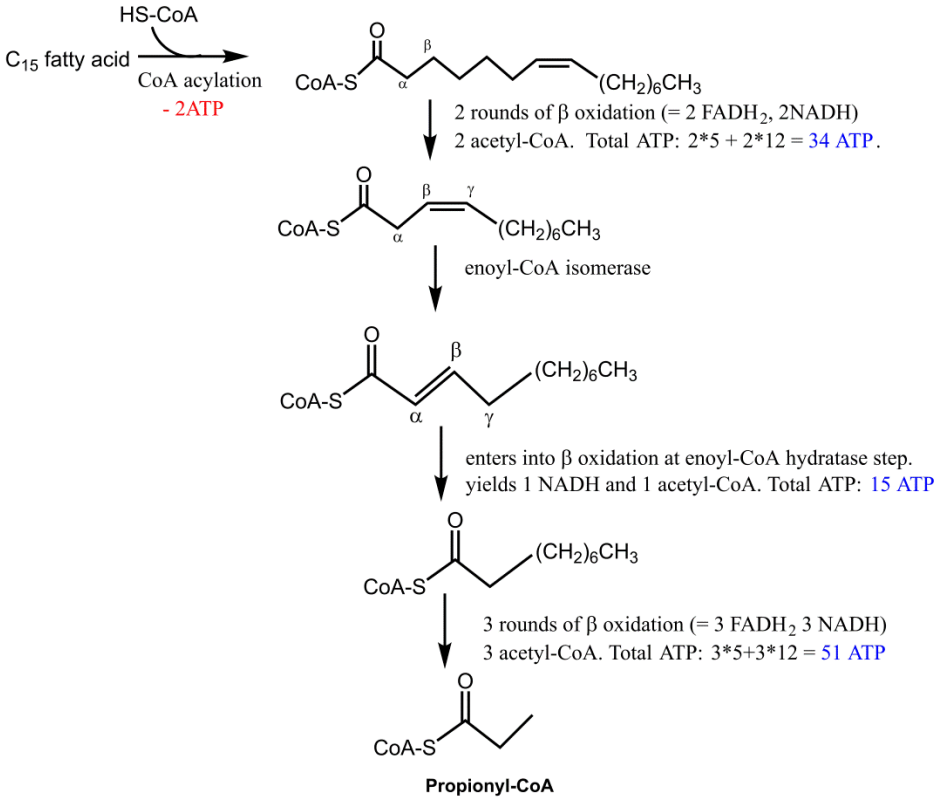
For the TAG given in this problem, there are two even-chain fatty acids. The 6-carbon FA will go through the β oxidation pathway twice and generate 3 acetyl-CoA equivalents.

ATPs from β oxidation pathway	**= 5 x 2**	**= 10**
ATPs from acetyl-CoA	**= 12 x 3**	**= 36**
ATPs required for FA activation		**= -2**
	Total ATPs	**= 44**

The 16 carbon FA will go through the β oxidation pathway 7 times and generate 8 acetyl-CoA equivalents.

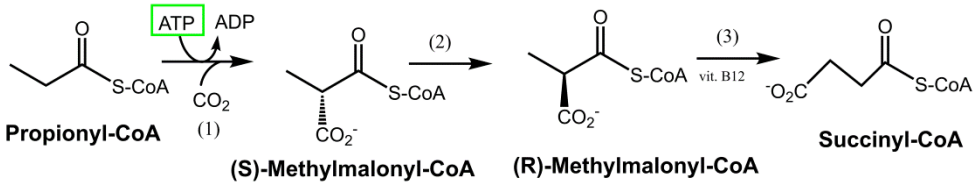
ATPs from β oxidation pathway	**= 5 x 7**	**= 35**
ATPs from acetyl-CoA	**= 12 x 8**	**= 96**
ATPs required for FA activation		**= -2**
	Total ATPs	**=129**

The odd chain, unsaturated fatty acid will be able to go two rounds through the β oxidation pathway before it is unable to do β oxidation due to the cis-β, γ double bond. For these two rounds of β oxidation, 17 x 2 = 34 ATP equivalents will be made. At this point, enoyl-CoA isomerase can convert the cis-β, γ double bond to a trans-Δ^2 double bond, and the FA can re-enter the β oxidation pathway at the enoyl-CoA hydratase step. Note that the unsaturation results in the loss of 2 possible ATP equivalents being generated (i.e: no $FADH_2$ is generated).

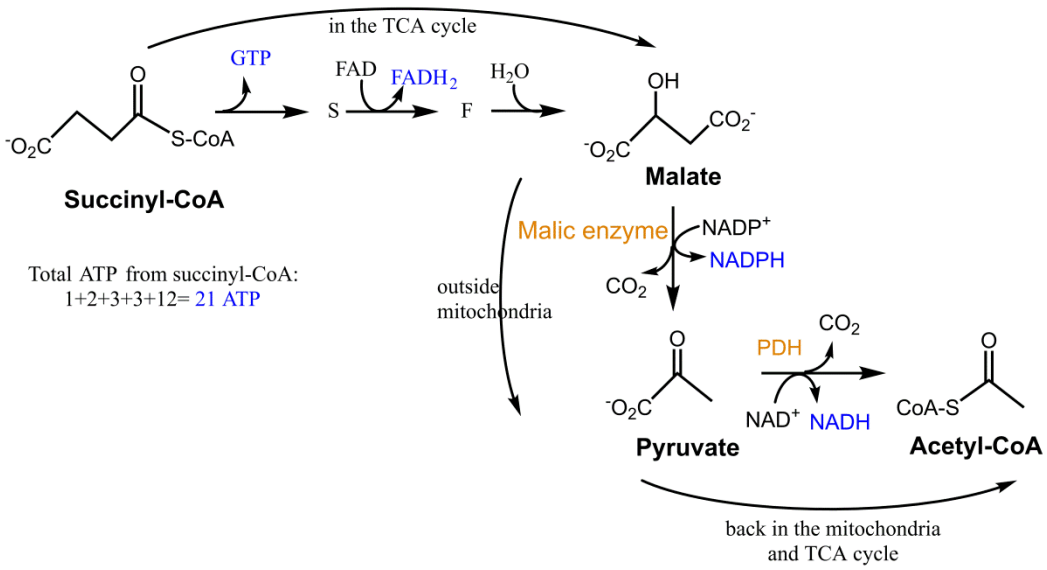


When propionyl-CoA is generated, it can be converted to succinyl-CoA through three steps:

1. **Carboxylation of the terminal methyl group (propionyl-CoA carboxylase, requires 1 ATP)**
2. **Racemization (methylmalonyl-CoA racemase)**
3. **Transfer of carboxyl group to propionyl-CoA (methylmalonyl-CoA mutase). This reaction is a rearrangement of the carbon skeleton, and requires adenosylcobalamin (vitamin B12).**



Succinyl-CoA will ultimately be converted to malate (via the TCA cycle). Keep in mind that addition of succinate (or any TCA cycle intermediate) will increase the rate of oxidation of substrates, but in order for the 4-carbon compound to contribute to net energy production, it must exit the mitochondrion and then re-enter as pyruvate. The most straightforward way to accomplish this task would be to exit as malate, and then be oxidized and decarboxylated by the malic enzyme. The ME generates NADPH, which is energetically equivalent to NADH.



Therefore, total yield of ATP from the C15 fatty acid is: -2+34+15+51-1+21 = 118 ATP.

The glycerol backbone of the TAG will also be oxidized. The glycerol can be converted to DHAP through two steps:

1. **Phosphorylation by glycerol kinase (requires 1 ATP)**
2. **Oxidation by glycerol-3-phosphate dehydrogenase (yields 1 NADH = 3 ATP)**

DHAP can then proceed through the glycolysis pathway (after conversion by TIM to GAP) and through PDH and TCA. The total yield of ATP from DHAP oxidation will be 20 ATP.

Total yield of ATP from glycerol = 22 ATP.

The overall yield of ATP from complete oxidation of this TAG is:

C6 FA :	**44 ATP**
C16 FA:	**129 ATP**
C15 FA:	**118 ATP**
Glycerol:	**22 ATP**
Total :	**313 ATP**

b. The six carbon FA yields a total of 44 ATP equivalents, while oxidation of one glucose yields 38 (or 36) equivalents. The reason for this result is that the carbons of glucose are at a higher oxidation state. The six-carbon FA starts in a more reduced state, so there is more energy that can be obtained by burning it (i.e. more electrons available for ET and OxPhos).

Problem 2

After adding the lipid in Problem 1 to a mammalian cellular extract, you note that the rate of evolution of CO_2 from glucose increases (note that I wrote "glucose"). You speculate that some kind of "catalyst" has been added to the system to cause this enhanced rate of CO_2 evolution.

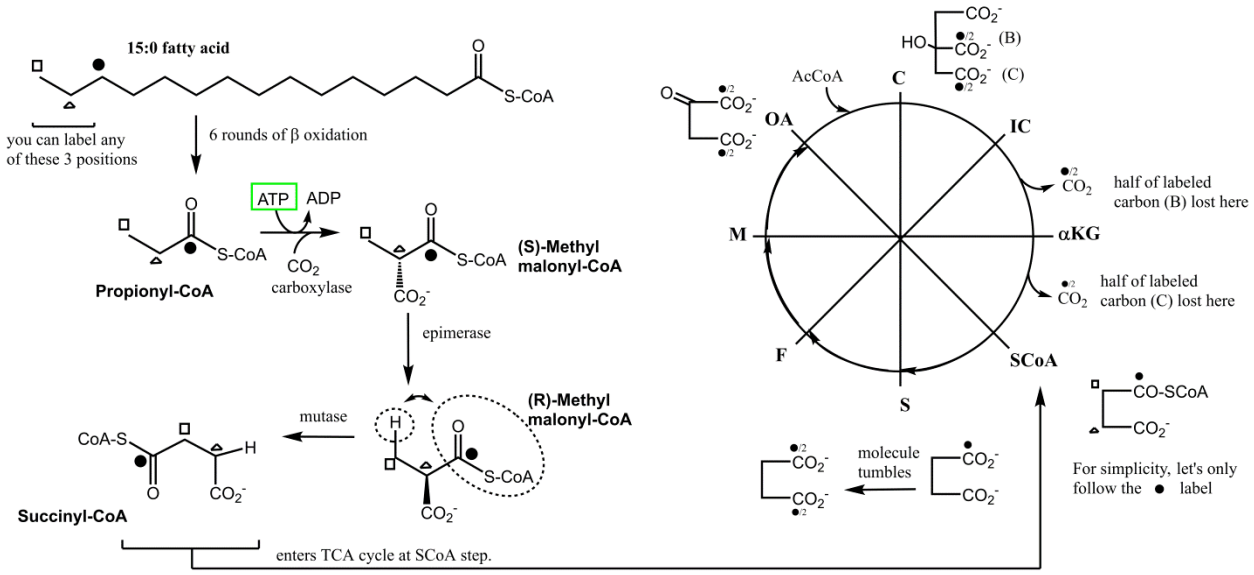
- A friend who aced 5.07 tells you that she thinks you can probe the effect by making one of the fatty acids in the lipid radioactive, putting the radioactive fatty acid into a metabolic extract, and then looking over short intervals of time at the labeling pattern in TCA cycle intermediates. Show each molecule of the TCA cycle and its anticipated labeling pattern through one turn of the cycle. Note that you need to design the compound you need to test your hypothesis. Imagine that you have the capability to synthesize any part of the molecule in Problem 1 with a ^{14}C label exactly where you want to put it.
- Another friend suggests that you use Pfizer's biotin carboxylase inhibitor, which knocks out the "catalytic effect."

Put together a pathway showing how the lipid in Problem 1 causes the catalytic enhancement of the TCA cycle. Draw out the chemical reactions in reasonable detail along the way. Use all of the data in the bullet points above.

Answer:

The key concept here is that the odd chain fatty acid (15:0) has the capacity to become succinate via the action of the biotin-containing enzyme propionyl CoA carboxylase, methyl malonyl CoA epimerase and methyl malonyl CoA mutase. The succinate increases the amount of all TCA cycle intermediates, thus increasing the catalytic power of the TCA cycle. Whether fatty acid or glucose is catabolized is not relevant … the key thing is that there is more catalyst and hence the overall rate of metabolism of anything in the cycle is increased.

Experiment to test hypothesis … you could label any of the distal three carbons of (15:0) to test your model.

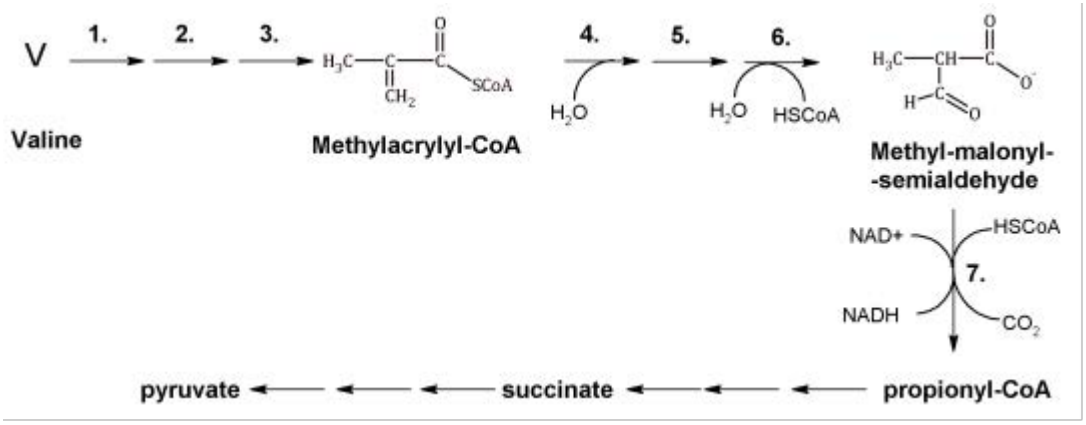


Pfizer's biotin carboxylase inhibitor would inhibit the first step in the conversion of propionyl CoA to succinate. Without supplemental succinate, the rate of processing of any intermediate by the TCA cycle would not be increased.

Problem 3

Amino acid catabolism is an important group of biochemical pathways that allows the use of amino acids as fuels. These pathways can convert any amino acids into intermediates of primary metabolism. These processes are very important in conditions of extreme starvation, when proteins are broken down into amino acids, which then can be metabolized to generate energy.

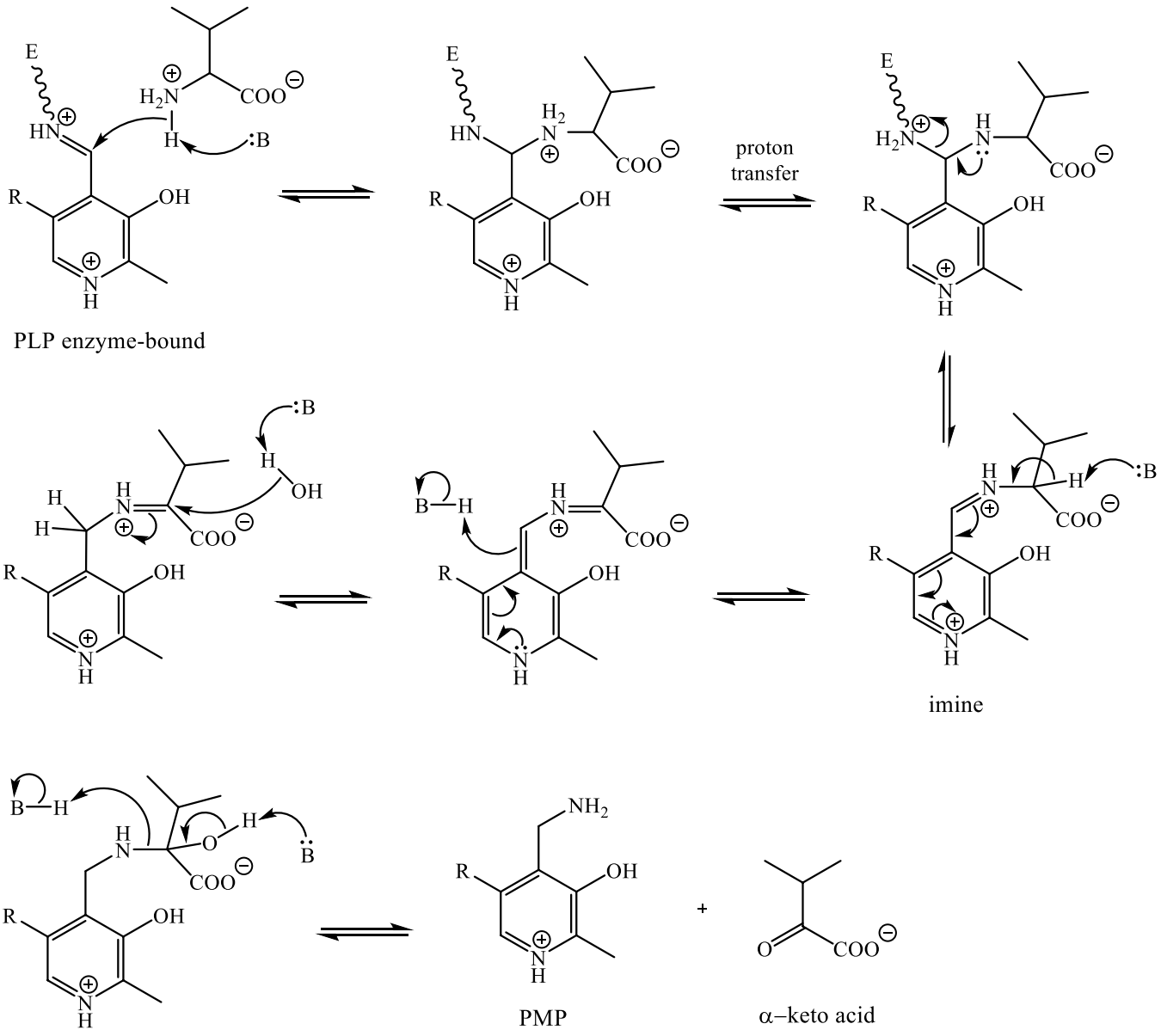
Degradation of branched chain amino acids occurs by reactions analogous to many of those you have seen in primary metabolism. The pathway for valine (one of the branched-chain amino acids) is sketched below.



a. The first step in degradation of almost any amino acids involves a transamination reaction. Indicate the cofactor required and write the mechanism.
b. Step 2 is the decarboxylation of an α-keto acid and involves a multienzyme complex that includes 3 proteins and 5 co-factors. This complex is very similar to the reaction discussed in class that connects glycolysis to the TCA cycle. Write a detailed mechanism for this transformation. Show the role of all cofactors.
c. Steps 3-5 are very similar to the steps in β-oxidation of fatty acids. Write the mechanism for these steps, indicating all cofactors required.
d. Step 6 hydrolyzes the SCoA group to generate methylmalonyl-semialdehyde. This compound is then oxidized and decarboxylated in step 7 by an enzyme called methylmalonyl-semialdehyde dehydrogenase (MSDH) to generate propionylCoA. MSDH has an active site cysteine and uses a mechanism similar to GAPDH. Propose a mechanism for this transformation.
e. To generate energy, propionyl-CoA is converted to pyruvate and fed into the TCA cycle. Write out the metabolic steps that accomplish this transformation. You do not need to show the mechanisms. Just draw each intermediate and indicate what cofactors are required.
f. Some of the steps in the conversion of propionylCoA to pyruvate take place outside the mitochondria. Indicate which ones and briefly explain why.
g. How much ATP can we generate by completely metabolizing valine to CO_2? (Assume each NADH generate gives 3 ATP, each FADH2 gives 2 ATP).
h. Is valine a glucogenic or ketogenic amino acid? Briefly explain your answer.
i. If valine was ^{14}C labeled at both α and β positions, indicate the metabolic steps in which $^{14}CO_2$ will be released.

ANSWER:

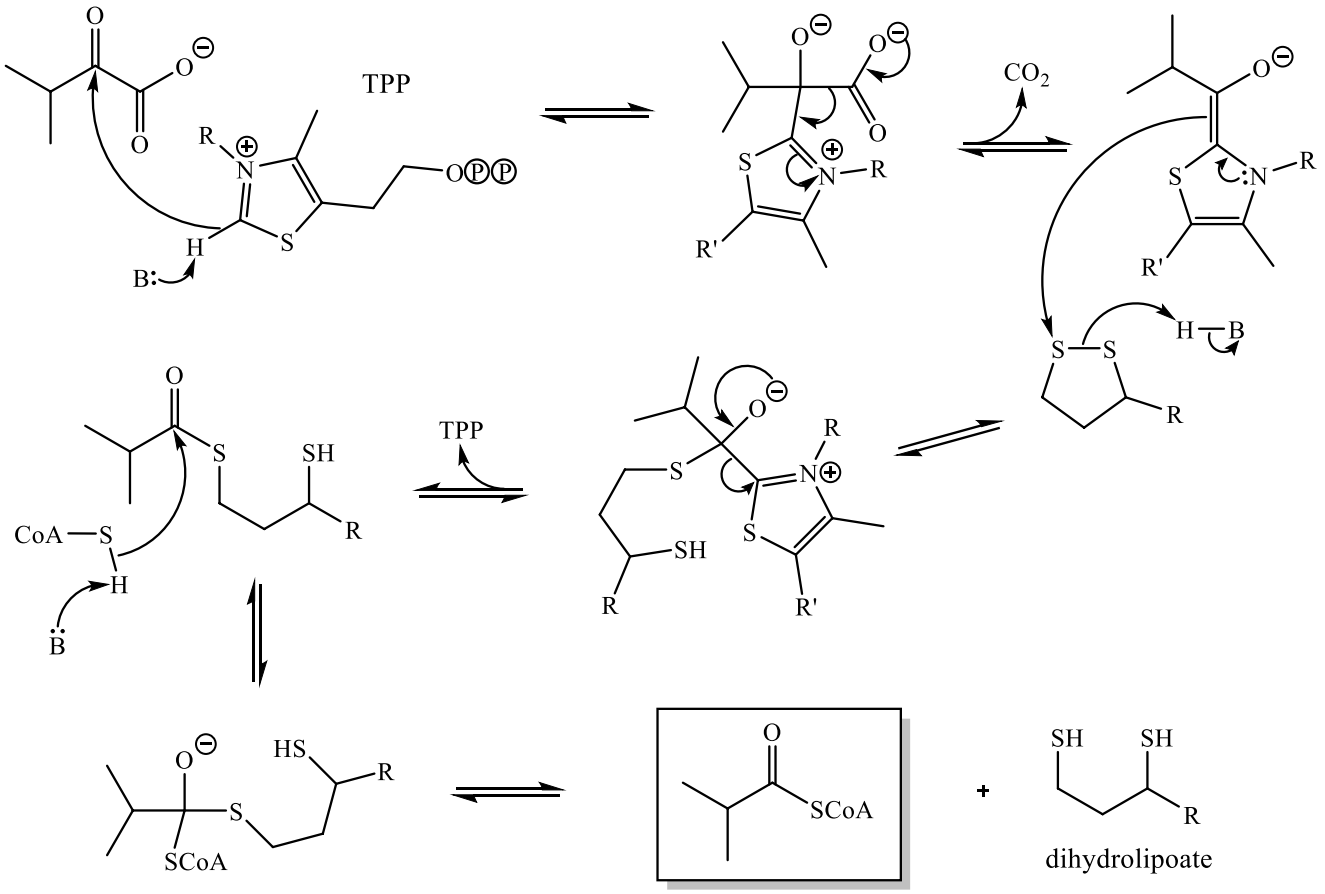
a. The transamination reaction mechanism is similar to the one discussed in class. It requires PLP cofactor, which is converted to PMP. To regenerate PLP, we need to convert an α-keto acid into an aminoacid (i.e., α-KG into Glutamate).

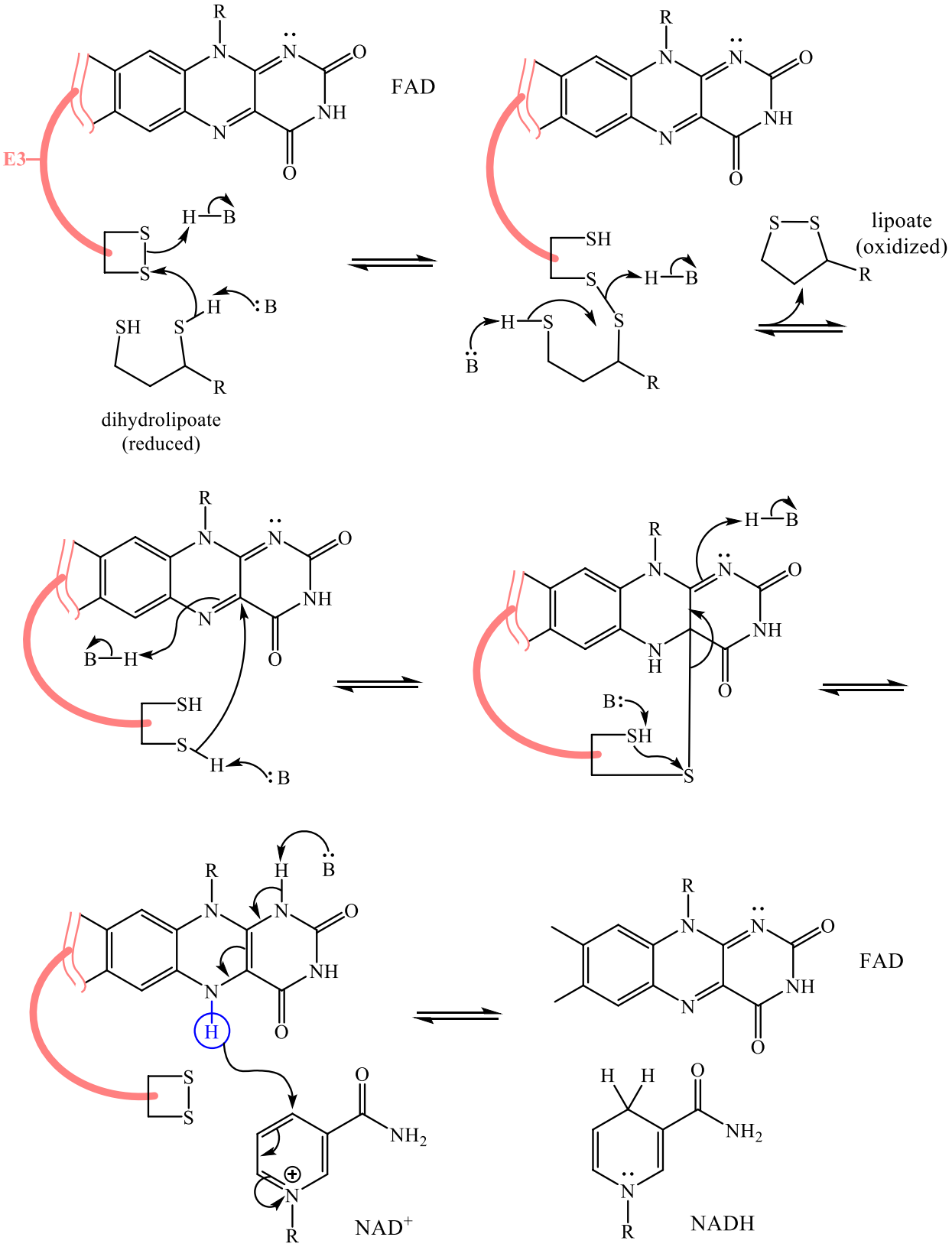


b. Step 2 is similar to pyruvate dehydrogenase (PDH) reaction. It involves the oxidative decarboxylation of an α-keto acid, generating a CoA thioester. The five cofactors

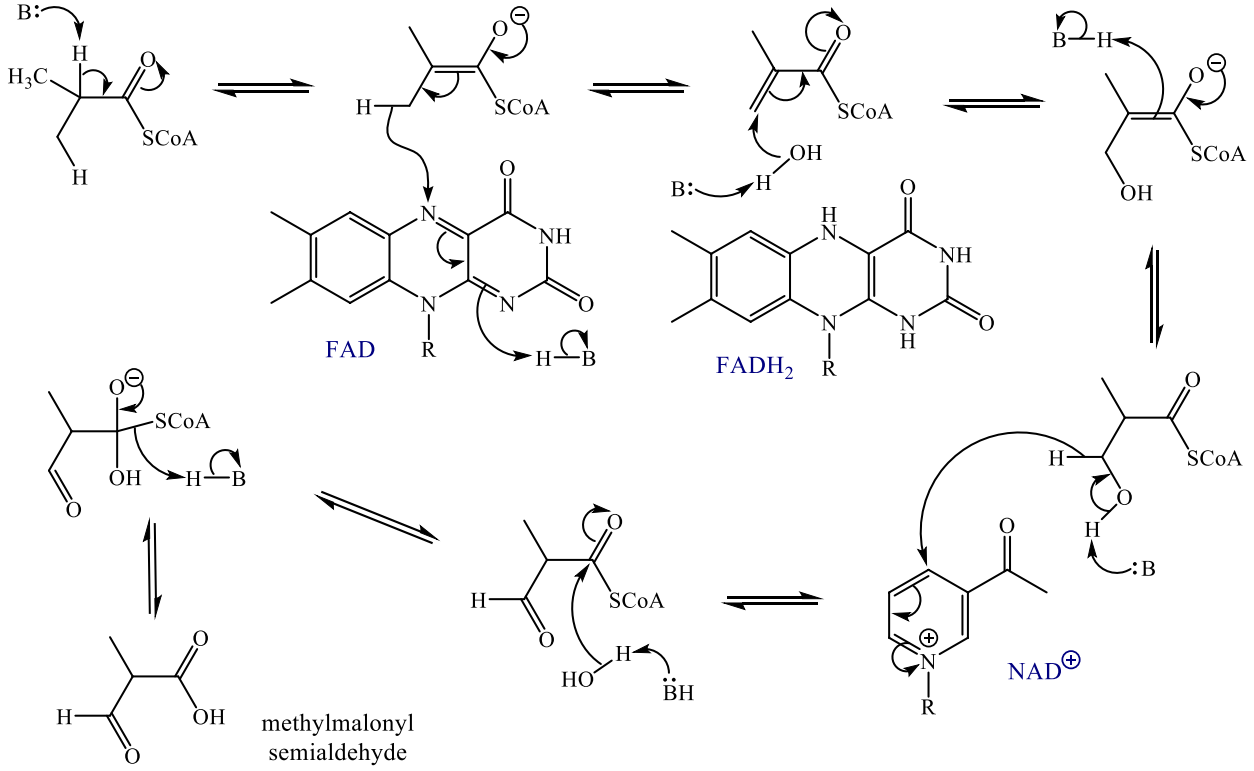
involved are: TPP, lipoate, HSCoA, FAD, and NAD^+. All the cofactors are regenerated in the course of the reaction, except NAD^+ which is converted to NADH.

The mechanism is similar to the one outlined in your books and notes.

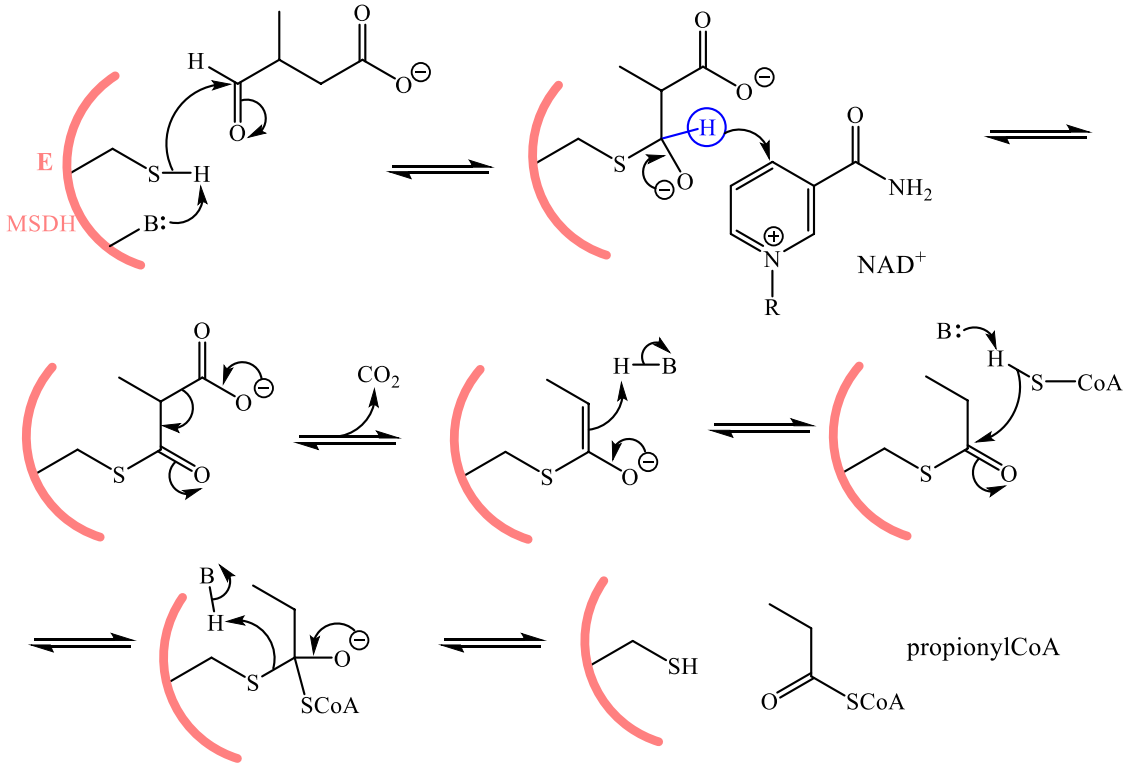



E3
FAD
dihydrolipoate
(reduced)
lipoate
(oxidized)
NAD+
NADH

c. These 3 steps are similar to the β-oxidation of fatty acids.

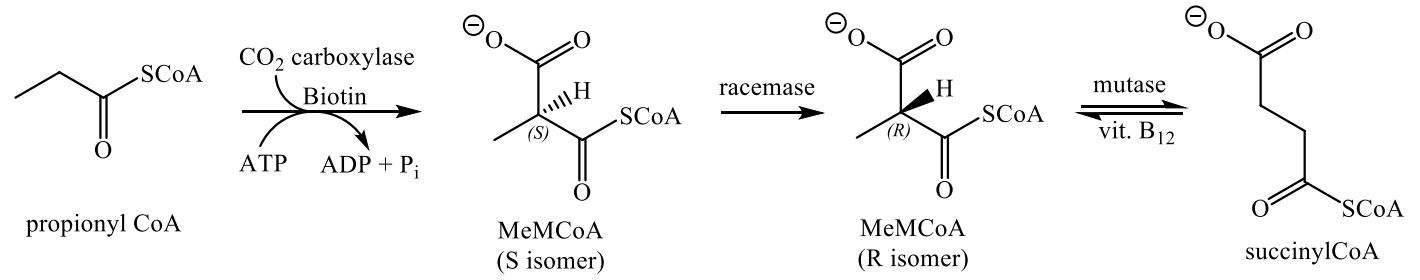


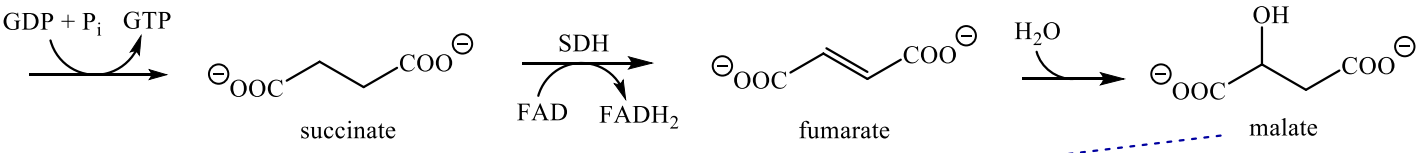
d. The mechanism is very similar to GAPDH, involving the formation of a thioester with the active site cysteine. Once the thioester is formed, the malonyl thioester derivative can lose CO_2 (a facile reaction for a β-keto acid).

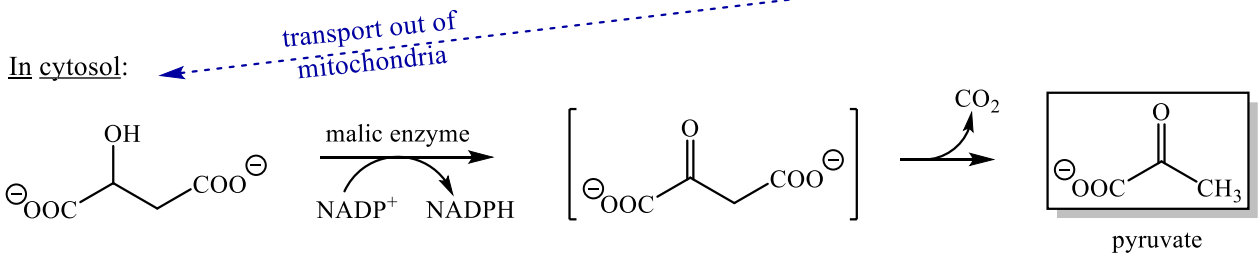


e. The pathway is outlined below. First, propionyl-CoA is carboxylated, then the carbon frame is rearranged to form succinylCoA. This can go through some steps of the TCA cycle up to malate. Malate goes into the cytosol where it can be converted to pyruvate by the malic enzyme.

In mitochondria:

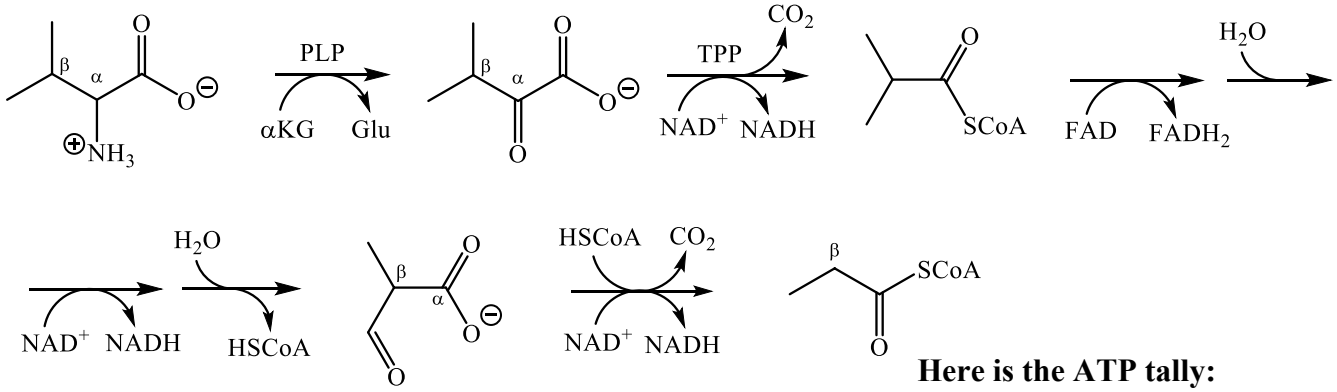






f. The previous outline also indicates where the steps are taking place. The malic enzyme is only cytosolic, so one of the intermediates in the pathway needs to go out of the mitochondria. Malate can use the shuttle to come out and be converted into pyruvate by the malic enzyme. Having the malic enzyme inside mitochondria would prevent regeneration of OA from malate and thus, it would make it impossible to run the TCA cycle.

g. The beginning of the pathway is summarized here:



Here is the ATP tally:

- **Valine to propionlyCoA generates: 3 NADH, 1 $FADH_2$**
- **PropionylCoA to pyruvate gives: 1 NADPH, 1 $FADH_2$ (also use 1 ATP, get 1 GTP).**

- **Pyruvate to CO_2 generates: 4 NADH, 1 $FADH_2$, 1 GTP.**

If we assume we get 3 ATPs from NADH (or NADPH) and 2 ATPs from $FADH_2$, then the total is: 8×3 + 3×2 + 1 = 31 ATPs.

h. Valine is a glucogenic aminoacid. The degradation of valine only generates propionylCoA, then pyruvate (or PEP), which can then be used in gluconeogenesis. Notice that valine, unlike isoleucine, does not generate directly any acetylCoA, so it is not ketogenic. Compare this to isoleucine, which generates both acetylCoA and propionylCoA; this makes isoleucine both ketogenic and glucogenic.

i. **The α carbon will be released as CO_2 in step 7, catalyzed by MSDH. The β carbon will end up as the second carbon in propionylCoA. When succinate is formed from succinylCoA, after the mutase reaction, the label will scramble between the two middle carbons. Then, it ends up equally distributed between carbons 2 and 3 in pyruvate. None is lost at the PDH step, so the label will be with equal probability in either of the two carbons of acetylCoA. If it is on the carboxyl group, it will be released completely as CO_2 after 2 rounds of the TCA cycle. If it is on the methyl of acetylCoA, it will be released 50% at a time starting from round 2 of the TCA cycle.**
All CO_2 releases in the TCA cycle happen at the ICDH and αKGDH steps.

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