Chapter 20 Answers
Citric Acid Cycle

1) Book Study exercises 1 and 2 Below see the book for question answers
For study exersize 1, the five reactions of the PDH complex are:
1 - pyruvate dehydrogenase (E1) decarboxylates pyruvate, releasing CO2 and leaving a hydroxyethyl group bound to its TPP proshetic group.
2 – Dihydrolipoyl transacetylase (E2) transfers the hydroxyethyl group from TPP to its lipoamide prosthetic group, producing acetyl-dihydrolipoamide.
3 – E2 then transfers the acetyl group to CoA, yielding acetyl CoA and a dihydrolipoamide group.
4 – Dihydrolipoyl dehydrogenase (E3) oxidizes the dihydrolipoamide group of E2 by a disulfide interchange with two E3 Cys residues
5 – The two reduced Cys residues are oxidized by NAD+ though the intermediacy of FAD, producing NADH and regenerating E3 (section 16-2B)

2) Which enzyme is not found in the mitochondrial matrix?
1. Pyruvate dehydrogenase
2. Malate Dehydrogenase
3. Succinate Dehydrogenase ****
4. Citrate Synthase

4) An amino acid feeds into the TCA through α-ketoglutarate and exits via oxaloacetate. How many ATPs are ultimately produced.

2 NADH, 1 FADH are produced and one GTP is formed. Assuming 100% conversion 9 ATP's are produced

5) Which enzyme catalyzes the following reaction?
Acetyl CoA + oxaloacetate -> CoASH + citrate

Citrate synthase

6) Draw the reaction catalyzed by α ketoglutarate dehydrogenase

see the book

7) Draw the reaction in the TCA cycle that requires FAD

see the book

8) Explain how the malate dehydrogenase reaction can occur when the change in Gibbs free energy is + 7.1K Cal

Consider the coupling of the MDH and Citrate synthase reactions. The relative concentrations of OAA is very low compared to malate when considering the reaction of MDH alone in vitro. However there is a tight association of the two enzymes in the mitochondria. That allows nearly all of the OAA formed to immediately be converted into citrate as long as there is Acetyl CoA present. This is possible due to the very exergonic reactions of citrate synthase.

9) Assign the co-enzymes involved in the pyruvate dehydrogenation reaction. Identify which complex it belongs to and the part in each reaction it plays. What are the important points of this reaction?
E1, E2 and E3. Know the reactions

10) Malonate is a potent inhibitor of succinate dehydrogenase. Look at the structures and explain why. Hint, look at the normal reaction and its product, fumarate and remember the mechanisms for inhibition that we learned about in the past.

CH_2-COO^-

CH_2 Malonate

CH_2-COO^-

The substrate for SDH is succinate. Malonate is very similar in structure or succinate. Because malonate only has one methylene group, only one H can be lost (consider the reaction and H+ lost by succinate). Malonate cannot be dehydrogenated and is therefore unreactive. Furthermore and very important this can be used to determine the reactions of the TCA.

11) Why doesn't citrate synthase hydrolyze acetyl CoA?

This is a condensation reaction not a hydrolysis. Hydrolysis would leave free acetyl groups, CoA and unreacted oxaloacetate.

12) Explain why O_2 doesn't play a direct role in the TCA cycle, yet with out the presence of O_2, the cycle does not appreciably proceed?

The O_2 is required for the oxidation of NADH and $FADH_2$, without replacing these reducing equivalents (substrates) the reaction can no longer continue.

13) Write a table of activators and inhibitors of the TCA cycle. What is the relationship between activation or inhibition and the energy state of the cell?

14) A runner needs a tremendous amount of energy during a long distance endurance race. Explain how the use of ATP by contracting muscles affects the use of the citric acid cycle.

This will regulate the cycle by altering the ATP/ADP ratio. Additionally, Acetyl CoA will be produced which will also activate the cycle by increasing pyruvate carboxylase.

15) Write a balanced equation for the citric acid cycle

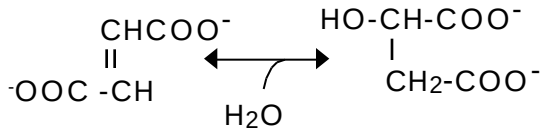
16) Follow the carbons of the TCA. Where are the carbons from acetyl CoA after one turn? After several turns of the cycle?

The carbons added at A-CoA are not lost during the first turn of the cycle. These are later lost in the two reactions in the second turn.

17) If asparate is produced by the transamination of α-ketoglutarate, which enzyme will be required to replenish the TCA cycle intermediates?

a) citrate synthase
b) malate dehydrogenase
c) alpha ketoglutarate dehydrogenase
d) pyruvate carboxylase ***
e) pyruvate dehydrogenase

18) The enzymatic reaction which follows is:



a) malate dehydrogenase
b) fumarase ***
c) succinate thiokinase
d) succinate dehydrogenase
e) phosphoenolpyruvate carboxykinase

19) The reaction above is a condensation

a) condensation
b) decarboxylation
c) dehydration
d) hydration
e) oxidation
f) substrate-level phosphorylation

20) Which enzyme/s are regulated by acetyl CoA (circle each correct answer)

a) citrate synthase ***

b) aconitase
c) pyruvate dehydrogenase ***
d) α-ketoglutarate dehydrogenase
e) pyruvate carboxylase ***

21) The conversion of α-ketoglutarate does not include which of the following cofactors:
a) lipoate
b) NAD+
c) thiamine
d) carbonyl phosphate

22) The activation of carboxy-biotin involves:
a) the formation of a carbonyl phosphate intermediate
b) AMP hydrolysis
c) the direct addition of CO_2 onto pyruvate
formation of GTP from oxaloacetate