

Summary of Chapter 16

1. Names of molecules and enzymes in citric acid cycle should be familiar.
2. Citric acid cycle takes place in the mitochondrion matrix.
3. Pyruvate is converted to acetyl-CoA before entering the citric acid cycle.
 - This reaction produces CO₂ and NADH from NAD⁺ in addition to acetyl-CoA.
 - Pyruvate dehydrogenase is a multienzyme complex, and catalyzes the oxidative decarboxylation reaction.
 1. Pyruvate dehydrogenase (E₁) has a bound coenzyme TPP and catalyzes decarboxylation.
 - E₁ is inactivated by phosphorylation with pyruvate dehydrogenase kinase.
 - E₁ is activated by dephosphorylation with pyruvate dehydrogenase phosphatase.
 - Insulin's signal activates the phosphatase. Thus, insulin works at both ends of glycolysis, i.e., reduces [glucose] in blood at the starting point and removes the end product (pyruvate) of glycolysis.
 2. Dihydropyridyl transacetylase (E₂) has a long lipoyllysyl arm (lipoamide group) composed of a lipoic acid covalently linked to the Lys residue with amide bond.
 - E₂ receives the hydroxyethyl group from E₁ with the oxidized lipoamide arm, catalyzes the acetyl-CoA formation, and releases the acetyl-CoA.
 - The lipoamide group is fully reduced by the acetyl-CoA formation, i.e., receives 2H⁺ and 2e⁻ in the catalytic reaction.
 3. Dihydropyridyl dehydrogenase (E₃) has a bound coenzyme FAD.
 - E₃ receives 2H⁺ and 2e⁻ from the reduced lipoamide group of E₂, and reduces NAD⁺ to NADH and H⁺.
4. Multienzyme complexes have catalytic advantages:
 1. Rate of a series of reactions are enhanced.
 2. Side reactions are minimized.
 3. Reactions can be coordinately controlled.
5. Coenzyme A is a carrier of acetyl and other acyl group, and the S~C is a "high-energy" bond which produces 31.5 kJ/mol energy by hydrolysis.
6. Acetyl-CoA is produced not only from glycolytic product (pyruvate) but also from fatty acids and amino acids.
7. Citric acid cycle reactions are summarized below:

	Reaction	Enzyme	Overall reaction	Substrates	Products
1	Acetyl-CoA + Oxaloacetate → citrate	Citrate synthase	Condensation reaction	H ₂ O	CoA
2	Citrate → isocitrate	Aconitase	Dehydration and hydration		
3	Isocitrate → α-ketoglutarate	Isocitrate dehydrogenase	Oxidative decarboxylation	NAD ⁺	NADH, CO ₂
4	α-ketoglutarate → succinyl-CoA	α-Ketoglutarate dehydrogenase	Oxidative decarboxylation	CoA, NAD ⁺	NADH, CO ₂
5	Succinyl-CoA → succinate	Succinyl-CoA synthetase	Substrate level phosphorylation	GDP	ATP, CoA
6	Succinate → fumarate	Succinate dehydrogenase	Dehydrogenation	FAD	FADH ₂
7	Fumarate → L-malate	Fumarase	Hydration	H ₂ O	
8	L-Malate → oxaloacetate	Malate dehydrogenase	Dehydrogenation	NAD ⁺	NADH

8. Aconitase contains a bound [4Fe-4S] cluster. The intermediate is flipped so that C2 and C3 are exchanged their positions.
9. Fluoroacetate is very toxic. Why?
10. α-ketoglutarate dehydrogenase is another multienzyme complex consisting of three enzymes, which are closely resemble to the pyruvate dehydrogenase multienzyme complex.

11. Hydrolysis of the “high energy” S~C bond of succinyl-CoA produces a “high energy” GTP from GDP. GTP is converted to ATP by nucleoside diphosphate kinase.
12. Malonate inhibits succinate dehydrogenase since it is structural analog of succinate.
13. The oxidation of alkane to alkene is sufficient to reduce FAD to FADH₂, but not enough to reduce NAD⁺ to NADH. The oxidation of alcohol to aldehyde or ketone produces enough energy to reduce NAD⁺ to NADH.
14. Succinate dehydrogenase is the only membrane-bound citric acid enzyme since the covalently bound FADH₂ is only oxidized by the electron transport chain reaction.
15. Although the oxaloacetate formation from L-malate is relatively high endergonic reaction, this reaction occurs, because:
 1. The [oxaloacetate] is very low at equilibrium.
 2. The subsequent reaction is highly exergonic hydrolysis of the “high energy” S~C bond of acetyl-CoA. This is a reason why acetyl-CoA rather than acetate enters the citric acid cycle.
16. Most metabolites of citric acid cycle are present in both cytosol and mitochondria. Thus, it is difficult to establish the rate-determining step(s), but citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase are the citric acid cycle's rate-controlling enzymes since the reactions catalyzed by these enzymes have relatively large negative free energy changes.
17. The citric acid cycle is largely regulated by:
 - substrate availability (since metabolites of citric acid cycle exit as precursors of bio-syntheses and re-enter as products of bio-degradation).
 - product inhibition (citrate inhibits citrate synthase; succinyl-CoA inhibits succinyl-CoA synthetase, and acetyl-CoA inhibits pyruvate dehydrogenase)
 - competitive feedback inhibition (NADH and ATP inhibit the rate-controlling enzymes (citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase)).
18. Ca²⁺ and ADP are activators of the citric acid cycle.
19. The major role of citric acid cycle in muscle cells is degradation of acetyl-CoA to produce bioenergies.
20. In the liver cells, citric acid cycle works to produce not only bioenergies but also precursors of various biosyntheses (glucose, lipids, amino acids and porphyrins). Thus, the citric acid cycle is amphibolic, i.e., both anabolism (synthesis of biomacromolecules from small precursors) and catabolism (degradation of biomacromolecules to energy poor end products, such as CO₂, NH₃, H₂O).
21. When the citric acid cycle intermediates are transported too much as precursors, the [oxaloacetate] becomes very low. In this case, oxaloacetate is directly synthesized from pyruvate and CO₂, in order to continue the citric acid cycle:

$$\text{Pyruvate} + \text{CO}_2 + \text{ATP} + \text{H}_2\text{O} \rightarrow \text{oxaloacetate} + \text{ADP} + \text{P}_i$$
22. Bioenergy molecules produced from one glucose through glycolysis, pyruvate dehydrogenesis and citric acid cycle are:

	ATP	NADH	FADH ₂
Glycolysis	2	2	0
Pyruvate dehydrogenesis	0	2	0
Citric acid cycle	2	6	2
Total	4	10	2

23. A complete oxidation of one acetyl group (-COCH₃ → 2CO₂) is four electron pairs process, i.e.,

$$\text{CoA-S~COCH}_3 + 3\text{H}_2\text{O} \rightarrow 2\text{CO}_2 + \text{CoA-SH} + 8\text{H}^+ + 8\text{e}^-$$
 - Three electron pairs are utilized to reduce three NAD⁺ to NADH and one electron pair is utilized to reduce one FAD to FADH₂.
 - The NADH and FADH₂ are reoxidized, and four electron pairs are sent to the electron transport chain to reduce two O₂ to 4H₂O, i.e., 2O₂ + 8H⁺ + 8e⁻ → 4H₂O.
24. Oxidations of NADH and FADH₂ produce ~3 ATP and ~2ATP, respectively. Thus, a complete oxidation of one glucose (C₆H₁₂O₆ → 6CO₂) produce 38 ATP (4 + 10 × 3 + 2 × 2).