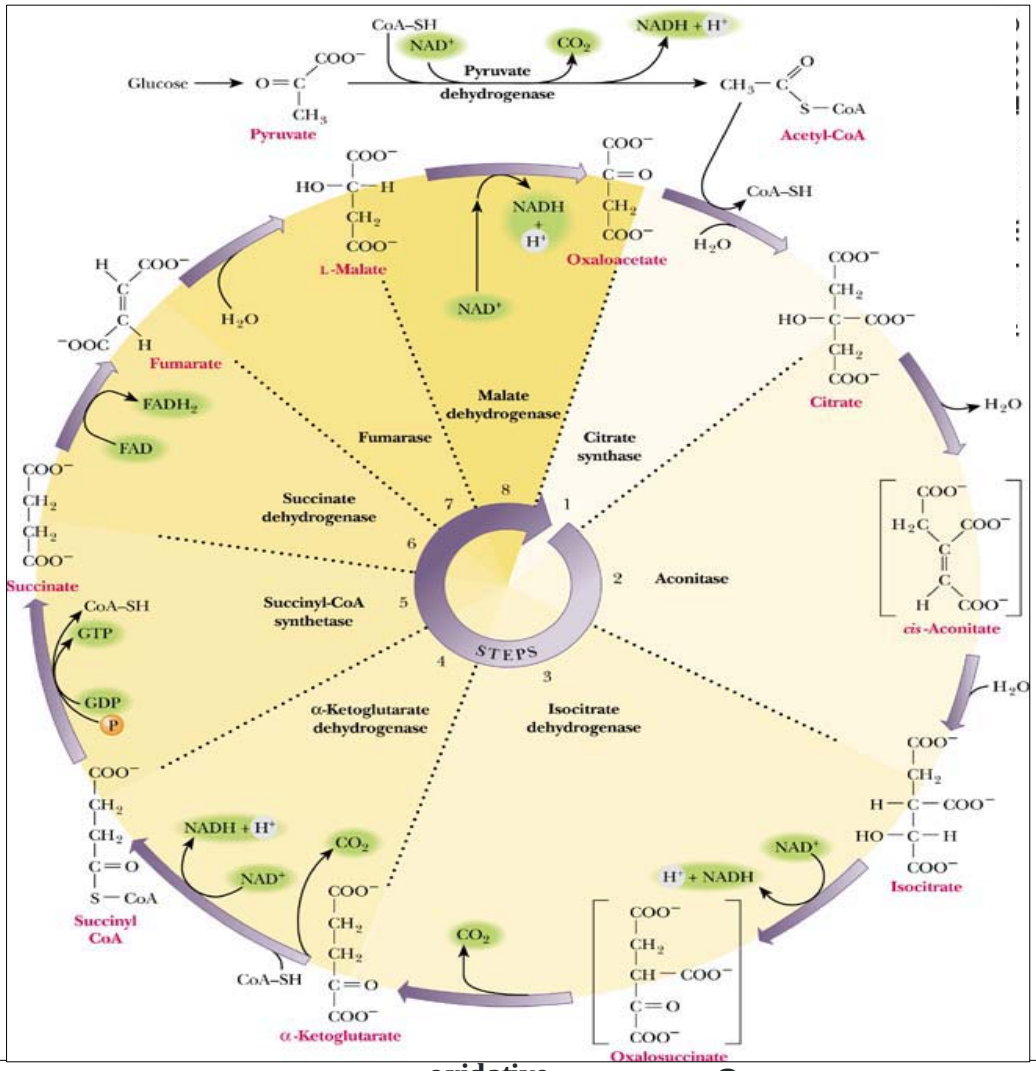
CH1010 Tutorial 7 Answers

1. Draw the **citric acid cycle** illustrating the **coenzymes**, **enzymes** and **substrate(s)** at each step.
   - Where does the acetyl coenzymeA and the oxaloacetate come from?



$$CH_3CCOO^- + NAD^+ + CoASH \xrightarrow{\text{oxidative decarboxylation}} CH_3CSCoA + CO_2 + NADH$$

Pyruvate **Acetyl-CoA**

The acetyl conenzymeA comes from oxidative decarboxylation of the 3C molecule pyruvate which is output from glycolysis (ie from glucose at the start). It may also come from amino acid degradation or fatty acid beta-oxidation.
If there is plentiful acetyl CoA some pyruvate is converted to oxaloacetate to provide substrate for step 1, the enzyme involved is pyruvate carboxylase.

2. Explain the process of **regulation** of the TCA cycle.

The citric acid cycle is controlled by three feedback mechanisms:

- citrate synthase :

Step 1 inhibited by ATP, NADH, succinyl-CoA and product inhibited by citrate. ATP and NADH are eventual products of the cycle (GTP is converted to ATP) hence this is product regulation. Controls the amount of citrate leaving the cycle to fatty acid, cholesterol and ketone body biosynthesis. Citrate is inhibitory to glycolysis and stimulatory to fatty acid biosynthesis the citrate concentration is a key in determining a cells metabolic direction. If there is a lot of energy around then this pushes citrate into biosynthesis and shuts down glycolysis to slow down the TCA cycle.

- isocitrate dehydrogenase:

Step 3 activated by ADP and NAD+, inhibited by ATP and NADH
This is the rate limiting step of the TCA cycle and so the energy state of the cell is highly regulated by this step which determines how fast the cycle goes.

- α-ketoglutarate dehydrogenase complex:

Step 4 activated by ADP and NAD+; inhibited by ATP, NADH and succinylCoA.
if NADH is high the enzyme is inhibited and alpha KG is diverted to glutamate for use in amino acid and protein biosynthesis.

3. **Pyruvate** which is a product of glycolysis may be converted into either acetyl-CoA or lactate.
   - Under what conditions does acetyl-CoA or lactate form?
   - Why is there a choice of two possible products for pyruvate metabolism?

Aerobic respiration.
Acetyl-CoA forms under aerobic conditions where oxidative decarboxylation transforms the 3C pyruvate into the 2C Acetyl-CoA. NAD^+ is required in this reaction and is also required in glycolysis. The energy efficiency is

With oxygen (Glycolysis+TCA+Respiration) 36ATP (13*36)/686 = 68%

Lactate fermentation.
Lactate forms under anaerobic conditions where a reduction transforms 3C pyruvate into the 3C lactate and NADH is oxidised to NAD^+. This is needed to keep glycolysis going as with oxygen around there is no ability of oxidative phosphorylation to transform NADH back to NAD^+.
Without glycolysis there is no energy being generated in the cell, with just glycolysis there is a very Small amount of energy being produced by Lactate fermentation. In muscle tissue this also builds up lactate and H^+ concentrations which quickly shuts down the operation of muscle cells.

Without oxygen (Glycolysis) 2ATP (13* 2)/686 = 4%

4. Draw a reaction scheme illustrating how ATP functions as a carrier of energy.

- Provide an example of how ATP is generated (ie a step in a reaction cycle).

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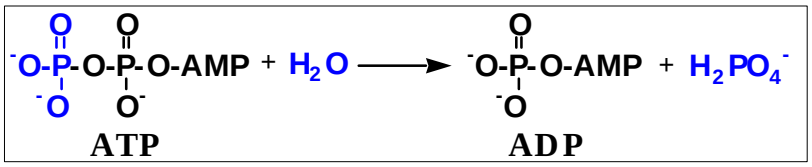
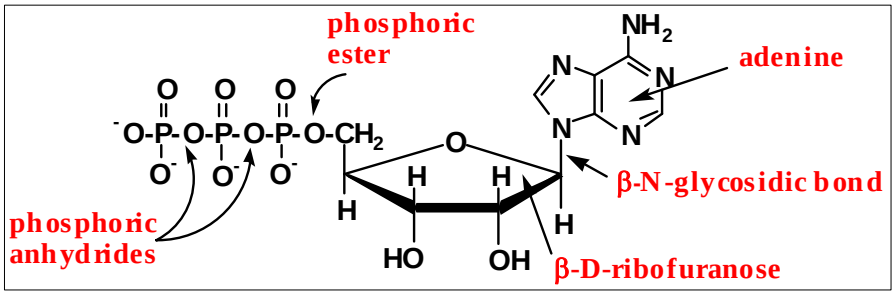
$\Delta G^0 = -30.5$ kJ mol^{-1} hydrolysis of ATP breaks a phosphorous anhydride bond liberating energy.





Pyruvate kinase is an enzyme which catalyses transfer of PO_4 to ADP(substrate) in glycolysis, Step 9. 2 cofactors Mg^{2+} and K^+ are found in the active site where catalysis occurs.