UNIT 10 METABOLISM-II

Structure

10.1 INTRODUCTION

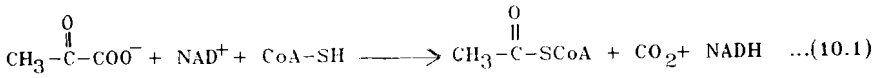
In the previous unit, you studied the metabolic breakdown of glucose and other sugars to pyruvate. Under anaerobic conditions pyruvate is subsequently converted into lactate in animal muscle or to ethanol in the yeast cells. These last reactions serve the purpose of regenerating NAD^+, i.e., reoxidising NADH, so that glycolytic process can be continued. In aerobic conditions however, NADH is reoxidised by transferring its electrons to oxygen through electron transport chain discussed in Unit 8. Under these conditions, pyruvate is metabolised further to CO_2 and H_2O by getting first converted into acetyl-coenzymeA. The acetate moiety of acetyl-CoA is oxidised via a cyclic metabolic pathway, called **tricarboxylic acid cycle** or **Kreb's cycle** or **citric acid cycle**. We would discuss this cycle in the present unit. Since pyruvate is also produced from an amino acid, alanine, and acetyl-CoA itself is a product of fatty acid degradation, the tricarboxylic acid cycle plays a central role in metabolism where the metabolism of carbohydrates, fats and proteins converge (Unit 9). Tricarboxylic acid cycle is directly related to the metabolism of several other amino acids through some of its metabolites. Further, it provides precursors for biosynthesis of some biomolecules. In this unit, we will study the conversion of pyruvate into acetyl-CoA, its oxidation via the tricarboxylic acid cycle as well as the metabolism of fatty acids. The next unit deals with the regulation of metabolism.

Objectives

After studying this unit, you should be able to:

- describe the reactions inolved in the conversion of pyruvate into acetyl-CoA,
- describe the steps involved in the tricarboxylic acid cycle,
- explain the central role of tricarboxylic acid cycle in metabolism including making precursors available for a variety of biomolecules,
- describe the pathways for the breakdown and biosynthesis of fatty acids, and
- explain the energetics of the above processes.

As mentioned in the introduction, under aerobic conditions pyruvate gets further metabolised through tricarboxylic acid (TCA) cycle. To enter the TCA cycle, pyruvate must first be converted into acetyl-CoA. For this, pyruvate is transported into mitochondria where it undergoes oxidative decarboxylation and condensation with coenzyme A (CoA-SH; see Unit 8 for structure).



This reaction is catalysed by an assembly of enzymes or a "multienzyme complex" called **pyruvate dehydrogenase complex.**

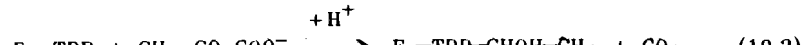
This complex consists of three distinct enzymes held together by strong noncovalent interactions. Consequently, they are isolated in the form of the complex rather than as three individual enzymes in contrast to many other enzymes which are isolated individually. Under some special conditions, the complex can be dissociated and the constituent enzymes isolated. On bringing the isolated enzymes together, they combine with a definite stoichiometry to reconstitute the initial complex thus suggesting that the forces holding them together must be very strong and that under the physiological conditions the complex is the more stable species than the individual enzymes existing independently. The three constituent enzymes and their respective prosthetic groups are listed in Table 10.1. In the complex the enzyme E_2 is present in the centre flanked by E_1 and E_3. In addition to the prosthetic groups of the constituent enzymes the complex as a whole requires NAD^+ as the coenzyme.

Table 10.1 : Constituent Enzymes of Pyruvate Dehydrogenase Complex

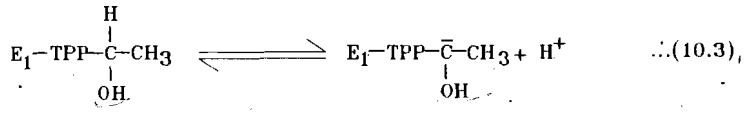
	Constituent enzyme	Abbreviation	Prosthetic group
1.	Pyruvate decarboxylase	E_1	Thiamine pyrophosphate (TPP)
2.	Dihydrolipoyl transacetylase	E_2	Lipoic acid
3.	Dihydrolipoyldehydrogenase	E_3	Flavin adenine dinucleotide (FAD)

In the complex the enzymes E_1, E_2 and E_3 act upon the pyruvate one by one. Individual reaction steps are outlined below.

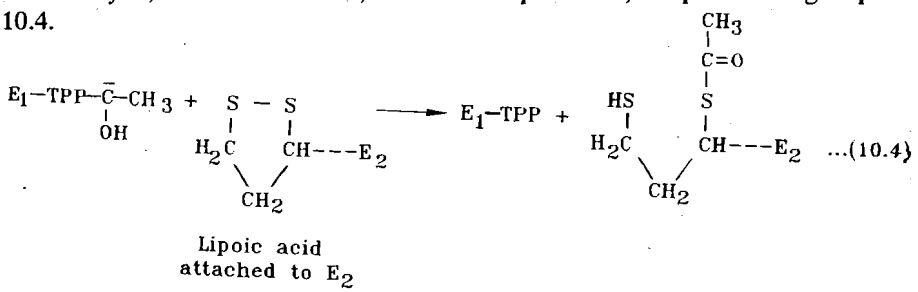
First of all, pyruvate undergoes decarboxylation catalysed by E_1. You may recall from Unit 9 that decarboxylation of pyruvate yields acetaldehyde. In the present case, the aldehyde is not released but remains covalently attached to thiamine pyrophosphate (TPP), the prosthetic group of E_1, Eq. 10.2.



The "hydroxy ethyl" group attached to TPP is not an alcoholic group but a "potential aldehyde", because it can readily lose a proton giving rise to a carbanion which is the "active aldehyde" form.

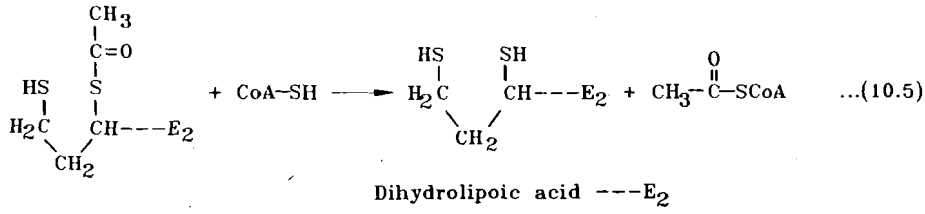


The potential aldehyde, or its active form, reacts with lipoic acid, the prosthetic group of E_2, Eq. 10.4.

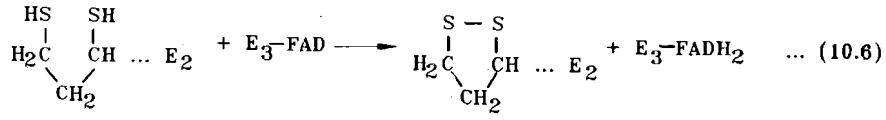


Lipoic acid attached to E_2

You can see that in this reaction, the potential aldehyde has been oxidised to the carboxylic acid state and the lipoic acid moiety has also been reduced to dihydrolipoic acid. Further, the acid has been retained in the enzyme complex in the form of a thiolester. In the next step, the acetyl group is transferred from the dihydrolipoic acid moiety to CoA-SH, Eq. 10.5.



Dihydrolipoic acid is reoxidised to lipoic acid moiety by reaction with E_3-FAD, Eq. 10.6.



Finally, the reduced prosthetic group of E_3, i.e., $FADH_2$, is reoxidised by NAD^+, Eq.10.7.

$$E_3 - FADH_2 + NAD^+ \longrightarrow E_3 - FAD + NADH + H^+ \quad ...(10.7)$$

Summation of Eq. 10.2 and 10.4 to 10.7 gives rise to Eq. 10.1. It may be pointed out that pyruvate has undergone several reactions, namely, decarboxylation, oxidation and condensation with the thiol group of CoA-SH. None of the intermediates is released into the medium. They remain attached to the enzyme complex and are transferred directly from one enzyme to another. This phenomenon is called **substrate channelling.**

A summary of these reactions is given in Fig. 10.1, in which the pyruvate dehydrogenase complex is shown by a box. Pyruvate, CoA-SH and NAD^+ enter the box as shown and CO_2, acetyl-CoA and NADH come out of it.

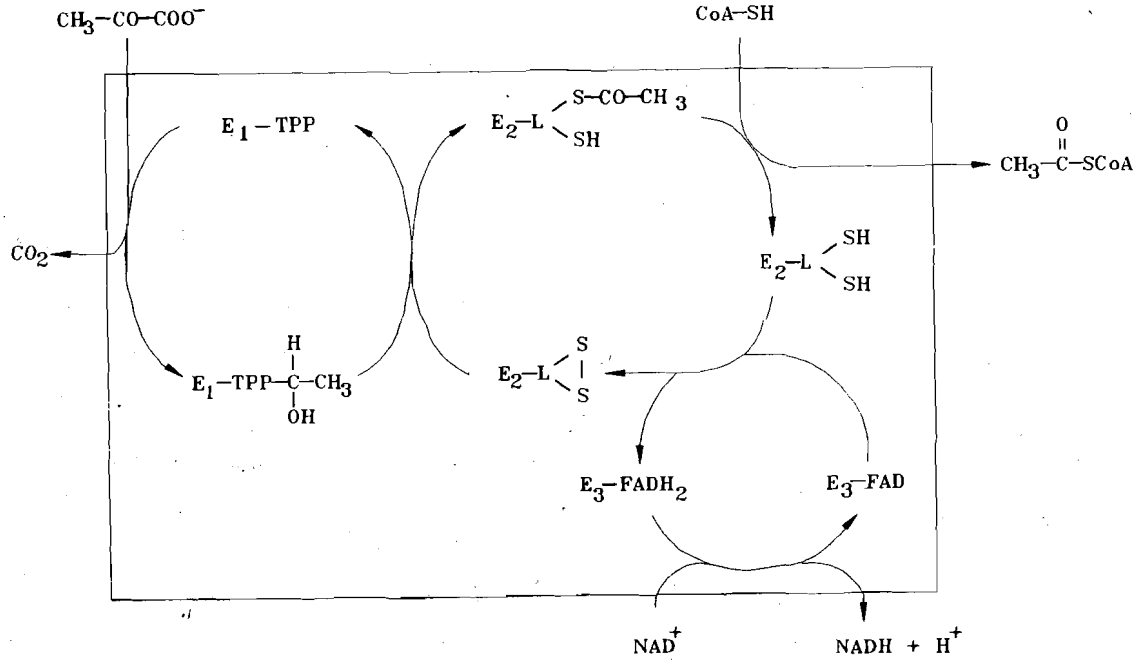


Fig. 10.1 : Summary of the reactions involved in the conversion of pyruvate into acetyl-CoA catalysed by pyruvate dehydrogenase complex (box). L stands for lipoic acid moiety given above.

It was mentioned in Unit 8 (Table 8.1) that a thiolester linkage is an energy rich linkage, the requisite energy coming from the oxidation of the aldehydic group (or of the potential aldehyde). It is partially conserved in the thiolester linkage, the rest of it being conserved in the form of reduced coenzyme, i.e., NADH. You would recall that this part of the energy can subsequently be recovered in the form of three ATP molecules when NADH is oxidised by the electron transport chain. Thus, this multienzyme complex is very efficient in terms of energy conservation. After studying the conversion of pyruvate to acetyl-CoA let us describe the fate of the acetyl-CoA which enters into the earlier mentioned TCA cycle. Before we proceed further try the following SAQ.

SAQ 1

Tick √ on right and x in front of wrong statements given below.

The conversion of pyruvate to acetyl-coenzyme A and CO_2

i) is catalysed mainly by lipoic acid.

ii) involves decarboxylation, oxidation and condensation reactions.

iii) is essentially irreversible.

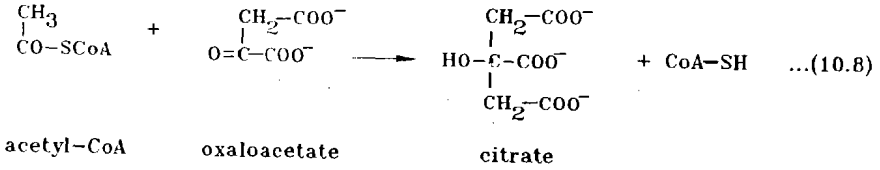
iv) forms a part of the tricarboxylic acid cycle.

10.3 TRICARBOXYLIC ACID CYCLE

Acetyl moiety of acetyl-CoA generated from pyruvate and by the degradation of fatty acids (to be taken up later, Section 10.4) is oxidised to carbon dioxide and water via a cyclic set of reactions, known as **citric acid cycle** or **Kreb's tricarboxylic acid (TCA) cycle**, after the name of the person who discovered it. As mentioned earlier, this cycle occupies a central position in the metabolic pathways, because its precursors, pyruvate and acetyl-CoA, may be derived from carbohydrates, fatty acids and some amino acids. A few other amino acids are degraded into metabolites which are intermediates in this cycle. In addition, some intermediates of the cycle serve as precursors in the biosynthesis of several biomolecules. Acetyl-CoA enters the cycle by reacting with oxaloacetate giving rise to citrate. This and the subsequent reactions of the cycle are described below.

10.3.1 Entry of Acetyl-CoA in the Cycle : Formation of Citrate

Acetyl-CoA reacts with oxaloacetate to give rise to citrate and coenzyme A is set free, Eq. 10.8. The enzyme catalysing this reaction is called **citrate synthase**. In earlier literature, it was referred to as the condensing enzyme.



You can see the similarity of this reaction with aldol condensation, i.e., the condensation of an α-CH group (next to CO) of acetyl-CoA with a carbonyl compound. This is the most commonly employed reaction in the physiological systems for establishing a new C-C linkage. You can compare the formation of fructose-1,6-bis-phosphate from glyceraldehyde-3-phosphate and dihydroxyacetone phosphate during gluconeogenesis (Unit 9).

10.3.2 Other Reactions of TCA Cycle

In the above reaction, a 4-carbon compound condenses with a 2-carbon acetate unit to form a 6-carbon compound, namely, citrate. The latter undergoes a series of reactions, summarised in Fig. 10.2 during which two molecules of CO_2 are lost and the original 4-carbon compound is regenerated.

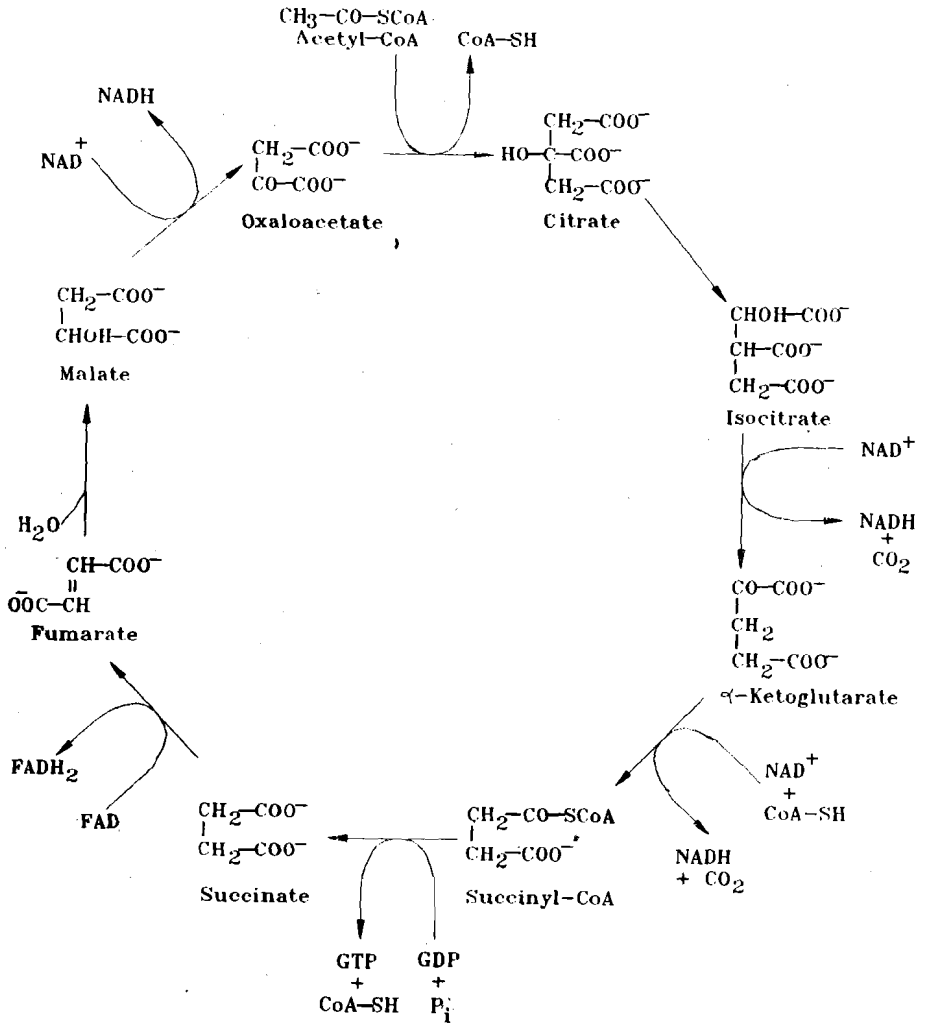
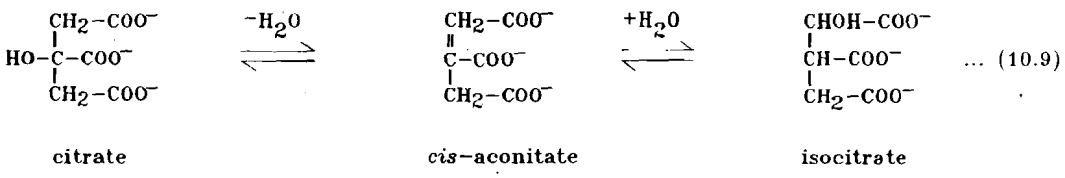


Fig. 10.2 : Summary of the reactions of tricarboxylic acid cycle

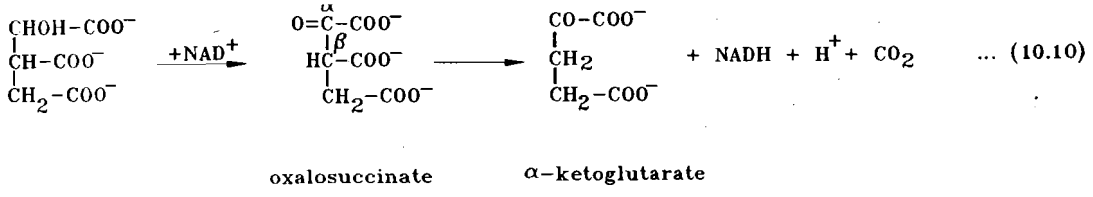
Aconitate, the dehydration product of citrate and isocitrate gives the enzyme its name, aconitase.

To start with, citrate is isomerised to isocitrate in the presence of the enzyme **aconitase**. The latter is an iron-sulphur protein in which iron is not complexed to any heme moiety. Therefore, it is also referred to as a nonheme-iron protein. It has been suggested that citrate undergoes a dehydration followed by a rehydration step with *cis* aconitate as an intermediate as shown in Eq. 10.9. This mechanism has, however, not been confirmed.



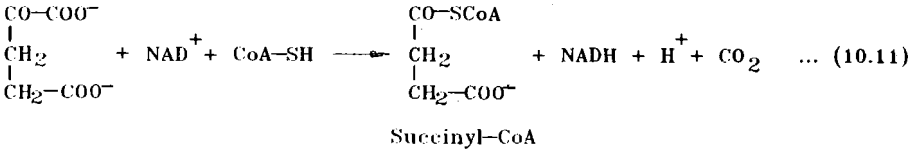
... (10.9)

Isocitrate reacts with NAD^+ in the presence of **isocitrate dehydrogenase** giving rise to α-ketoglutarate, a 5-carbon compound. This is achieved in two steps. In the first step the secondary alcohol is oxidised to ketone while the second step inolves β-decarboxylation.

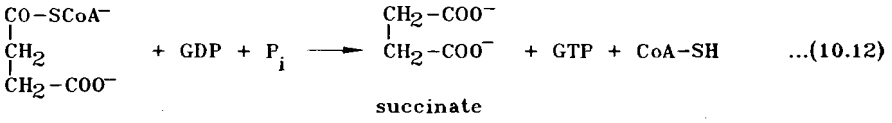


The loss of CO_2 helps push this reaction to completion towards right hand side.

The next reaction takes place in the presence of another multienzyme complex, namely **α-ketoglutarate dehydrogenase complex.** This complex is very similar to pyruvate dehydrogenase complex and so is the nature of the catalysed reaction as can be seen by comparing Eq. 10.11 with Eq. 10.1.

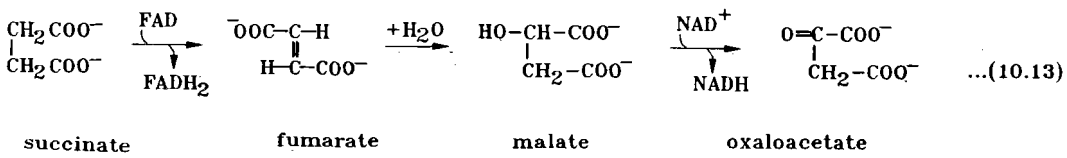


You may recall that the thiol-ester bond, which is present in succinyl-CoA, is an energy-rich linkage. This energy is recovered in the form of a pyrophosphate linkage of a nucleosidetriphosphate, GTP, in the next reaction which is catalysed by **succinyl-CoA synthetase** (name derived from the reverse reaction of Eq. 10.12).



You see that two molecules of CO_2 have been lost, one each in reactions of Eq. 10.10 and 10.11. Consequently, a 4-carbon acid has been formed. The next three reactions help to convert succinate into oxaloacetate, the original 4-carbon compound, which will complete the cycle and enable another molecule of acetyl-CoA to be taken up.

Succinate undergoes successively an oxidation, hydration and another oxidation reaction. In the first reaction, catalysed by **succinate dehydrogenase**, two hydrogen atoms are transferred from succinate to flavin adenine dinucleotide (FAD) to form reduced coenzyme ($FADH_2$) and an unsaturated acid, fumarate. It may be pointed out here that nature employs flavin nucleotide coenzymes wherever a saturated organic compound, generally an acid or its derivative, is to be converted into an unsaturated compound by removal of two hydrogen atoms and introduction of a C= C double bond. On the other hand, pyridine nucleotide coenzymes, NAD^+ or $NADP^+$, are utilised for the oxidation of alcoholic or aldehyde groups. Fumarate adds a molecule of water in the presence of fumarase to form malate and the latter is oxidised by NAD^+ to oxaloacetate in the presence of **malate dehydrogenase**. These three reactions are represented below:



Some of the intermediates of this cycle are involved in the metabolism of other biomolecules. The implications of these links will be discussed later in this unit. First try to answer the following SAQ.

SAQ 2

Tick √ the correct answer.

a) For continuity, the TCA cycle requires the regeneration of:

i) pyruvic acid

ii) oxaloacetate

iii) citrate

iv) malate

b) Before entering the TCA cycle, pyruvate gets converted to:

i) acetyl-CoA

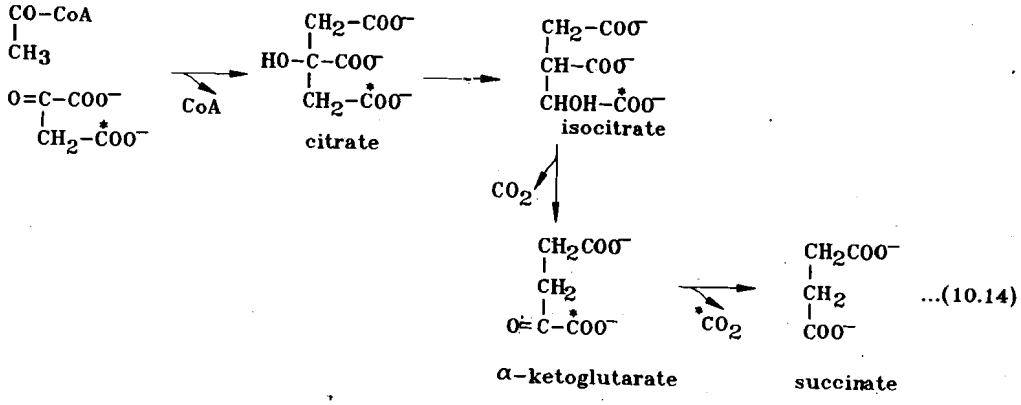
ii) citrate

iii) ethanol

iv) oxaloacetate

10.3.3 Stereochemistry of the TCA Cycle

The stereochemistry of interaction between enzymes and a certain type of substances was generalised by the application of isotopic tracer techniques to the TCA cycle. For example, if the carboxyl carbon farthest from the keto group of oxaloacetate is labelled with ^{14}C, the entire radioactivity is found to be present in α-ketoglutarate and is recovered as CO_2 in the next step, i.e., in the reaction of Eq. 10.11. The resulting succinate (Eq. 10.12) is completely devoid of any radioactivity. These results can be explained only if the sequence of reactions from citrate to succinate proceeds as depicted below:



You would see that in the symmetrical citrate molecule, the two CH_2-COO^- groups are chemically identical. There should have been equal chances of either one of them being involved in the conversion of citrate into isocitrate. If that were so, only half of the radioactivity should have been recovered as CO_2 and the remaining half should have been present in succinate. The experimental observation that the entire amount of radioactivity is recovered as CO_2 suggests that the enzyme aconitase somehow distinguishes between the two chemically identical CH_2-COO^- groups. Ogston pointed out that this was possible if three groups of the substrate were to be bound to three specific sites, or groups, of the enzyme as explained in Fig. 10.3.

Compounds, like citrate, having the general formula $CXYH_2$ are referred to as prochiral which can be transformed into chiral molecules, e.g., CXYZH, in a single step. Two isomeric chiral molecules can be obtained depending on the specific H atom which is replaced by Z. In the enzyme-substrate complex of the type depicted in Fig. 10.3, the two CH_2—COO^- groups are no longer chemically identical because one of them is bound to the enzyme and the other is exposed. They cannot interchange their positions because of the specific binding of the other two groups, namely, OH and COO^-, to their respective sites. Many other cases of distinction between chemically identical groups in prochiral molecules are encountered in biochemical reactions.

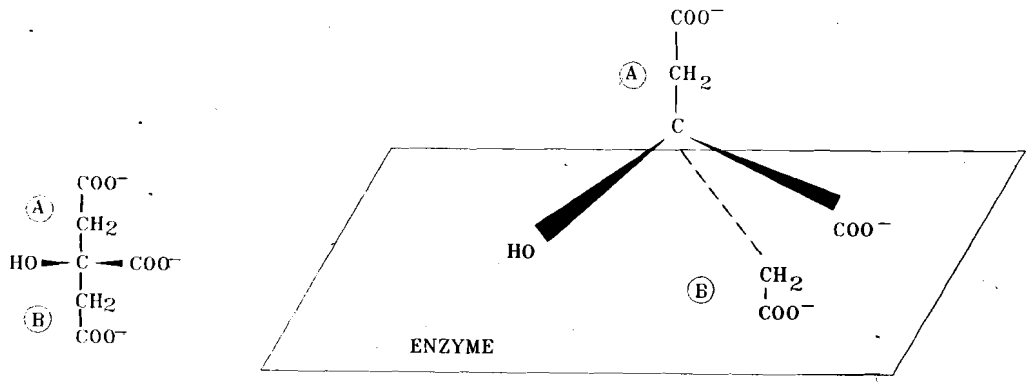


Fig. 10.3 : Postulated structure of citrate-aconitase complex. The enzyme active site has specific complementary sites for binding OH, COO^- and CH_2-COO^- groups. The OH and COO^- groups cannot interchange their positions. Consequently, only the CH_2-COO^- marked B in the structure of citrate can bind to the active site. The CH_2-COO^- group marked A cannot be bound in the given geometry of the active site, because it will displace the OH and COO^- groups from their respective specific sites.

10.3.4 Stoichiometry and Energetics of TCA Cycle

From Fig. 10.2 or by summing up the reactions of TCA cycle, we can arrive at its stoichiometry which is given below:

$$CH_3CO - SCoA + 3NAD^+ + FAD + GDP + P_i \rightarrow 2CO_2 + 3NADH + FADH_2 + GTP + CoA - SH \quad ...(10.15)$$

In this equation, water molecules and protons have been left out. It was shown in Unit 8, that oxidation of each NADH molecule via the electron transport chain is coupled to the synthesis of three molecules of ATP. Similarly, oxidation of each $FADH_2$ molecule causes the synthesis of two ATP molecules. Further, GTP is equivalent to and can be converted into ATP by the following reaction.

$$GTP + ADP \longrightarrow GDP + ATP \quad ...(10.16)$$

Thus, a total of (3 x 3) + (2 x 1) + 1 = 12 ATP molecules are synthesised for the oxidation of the 2-carbon acetyl moiety of acetyl-CoA via the TCA cycle. If we consider the complete oxidation of one molecule of glucose via glycolysis, conversion of the resultant two pyruvate molecules into two molecules of acetyl-CoA and their oxidation through the TCA cycle, the energy balance sheet works out as follows:

Reaction	ATP Synthesised	Reduced coenzymes formed
Glucose → 2 pyruvate (glycolysis)	2	2NADH
2 Pyruvate + 2CoA → 2acetyl - CoA + $2CO_2$ (pyruvate dehydrogenase complex)	-	2NADH
2 Acetyl - CoA → $4CO_2$ + 2CoA (TCA cycle)	2	6 NADH + 2 $FADH_2$
Total : Glucose → $6CO_2$	4	10NADH + 2$FADH_2$

Considering that each NADH is equivalent to 3 ATP and each $FADH_2$ is equivalent to two ATP, the total generation of ATP works out to be 38 molecules of ATP for each molecule of glucose oxidised. It may be noted that only two ATP molecules are formed during glycolysis and the remaining thirtysix are produced on the oxidation of two molecules of pyruvate. Further, major part of ATP synthesis (about 90%), takes place on the reoxidation of reduced coenzymes by the electron transport chain.

The overall efficiency of energy conservation, i.e., utilisation for ATP synthesis, is very high. Complete oxidation of glucose under standard conditions liberates 3085 kJ of free energy per mole. Free energy utilised for the synthesis of ATP from ADP and P_i under

standard conditions works out to be equal to $38 \times 30.5 = 1159$ kJ. This corresponds to $1159 \times 100/3085 \approx 37.5\%$ efficiency. The actual efficiency is much higher, because the conditions prevailing in the cell are different from standard conditions (see Unit 8).

You may recall that if glucose unit for oxidation is derived from glycogen, the number of ATP molecules synthesised during glycolysis will rise to three. Consequently, complete oxidation of glucose unit of glycogen will give rise to the synthesis of thirtynine ATP molecules.

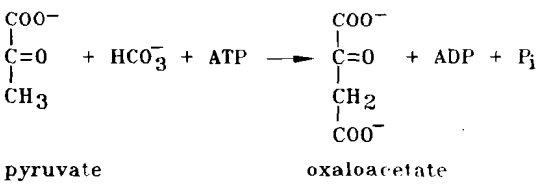
Having studied the steps, reactions, the stoichiometry and energetics of TCA cycle, let us try to understand its significance in metabolism.

10.3.5 Central Role of TCA Cycle

Besides bringing about the oxidation of acetyl moiety of acetyl-CoA to carbon dioxide, a catabolic function, TCA cycle also provides precursors for the biosynthesis of several other compounds, i.e., it performs an anabolic function. Therefore, it is often referred to as an **amphibolic** pathway. Let us study how this cycle is able to accomplish this.

It was mentioned in Unit 9 that catabolic, i.e., biodegradative, pathways are convergent. Some common metabolites are formed from a variety of starting materials, or nutrients, and these metabolites are further degraded by a common pathway. You saw the formation of pyruvate from carbohydrates and amino acids and that of acetyl-CoA from pyruvate and fatty acid. The acetyl-CoA is oxidised via the TCA cycle. Several amino acids, related to glutamate, and aspartate, are metabolised by their conversion into α-ketoglutarate and oxaloacetate, respectively, both of which are intermediates of this cycle. Some other intermediates of the cycle, e.g., succinate, are precursors of other biomolecules. These conversions illustrate the convergent nature of catabolism and the central role played by the TCA cycle.

Oxaloacetate and other metabolites of the TCA cycle have a "catalytic" role in the overall reaction (Eq. 10.15) in the sense that they are regenerated on the completion of the cycle. Their concentrations must be maintained at optimum levels to ensure an efficient running of the cycle and, therefore, of the metabolic breakdown of carbohydrates, fats and proteins. When some metabolites of this cycle are drawn as precursors for the synthesis of other biomolecules, their concentrations decrease. As a consequence, the concentrations of all the metabolites of the cycle are decreased because of the cyclic nature of the sequence of reactions. This leads to slowing down of the cycle and accumulation of acetyl-CoA and a decrease in the rate of ATP generation. It becomes necessary, therefore, to replenish some of the intermediates. One such replenishment reaction, significant in mammals, is the formation of oxaloacetate from pyruvate, CO_2 and ATP catalysed by pyruvate carboxylase (Eq. 10.17). Recall that this reaction is also involved in the conversion of pyruvate into phosphoenolpyruvate during gluconeogenesis (Unit 9).



pyruvate oxaloacetate ...(10.17)

Replenishment of any one intermediate of the cycle will increase the concentrations of all the metabolites of the cycle as explained above. The process of replenishment is called **anaplerosis** (meaning to "fill up").

Note that the major source of pyruvate in animal body is the glycolytic breakdown of carbohydrates. Availability of pyruvate is critical for anaplerosis and, therefore, for the efficient running of TCA cycle. As we will see later in this unit, fats are degraded to acetyl-CoA and the latter is normally metabolised via the TCA cycle. Thus, carbohydrates indirectly help in the catabolism or "burning" of fats in the body cells. This explains the necessity of including a certain amount of carbohydrates in the diets of persons who are trying to lose weight by "burning" some of the body fats. It has been

estimated that about one-fifth of the calorie, or energy, requirement of such persons must be provided as dietary carbohydrates. This ensures adequate supply of pyruvate which helps maintain optimum levels of TCA cycle intermediates. Metabolic consequences of the accumulation of acetyl-CoA, due to slowing down of TCA cycle will be described in Unit 11.

SAQ 3

Complete the statement by choosing a correct answer:

a) The number of ATP molecules produced by the oxidation of one molecule of pyruvate via TCA cycle is .....

   i) 2

   ii) 4

   iii) 10

   iv) 15

b) The TCA cycle is said to have a central role in metabolism because:

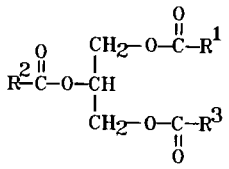
   i) it has a central place in the metabolism.

   ii) it has a cyclic nature.

   iii) it helps both in synthesising biomolecules (anabolism) and in catabolic pathways.

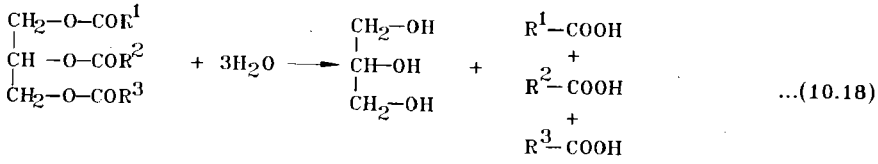
   iv) several amino acids are metabolised through this cycle.

10.4 METABOLISM OF FATS



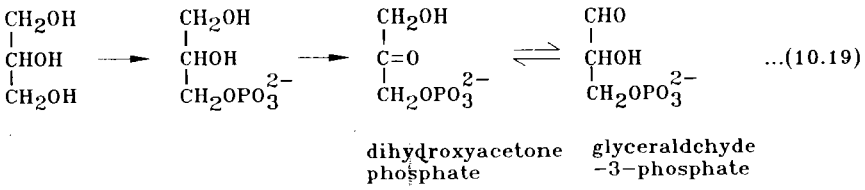
triacylglycerol

You know that oils and fats are major energy reserves of living beings including humans. You have studied their structure in Unit 3 of Block 1. They are esters of glycerol with fatty acids, i.e., they are triacylglycerols. The three acids, namely, R^1-COOH, R^2-COOH and R^3-COOH, may be identical or different. Major differences between the fatty acids lie in (i) the chain length of the alkyl groups, R^1, R^2 and R^3 and (ii) the presence and position of double bonds in these groups. The triacylglycerols are hydrolysed in the cytoplasm. The reaction, which takes place in more than one steps, is catalysed by various lipases, secreted into the intestinal lumen.



...(10.18)

Glycerol is phosphorylated and oxidised to dihydroxyacetone phosphate which is isomerised to glyceraldehyde-3-phosphate, Eq. 10.19 and then degraded via the glycolytic pathway (Unit 9), TCA cycle (Unit 10, Section 10.3) and electron transport chain (Unit 8).



dihydroxyacetone phosphate

glyceraldehyde-3-phosphate

...(10.19)

Each fatty acid is converted into the corresponding acylcoenzyme-A and transported into the mitochondrial matrix where it is degraded. In this section, we will discuss the

metabolic breakdown of saturated fatty acids only. The metabolism of unsaturated fatty acids and the mechanism of transport of acyl-CoA from the cytoplasm to the mitochondrial matrix will not be discussed.

10.4.1 Conversion of Fatty Acids into Acyl-CoA

Free energy stored in the pyrophosphate linkage of ATP is utilised to drive the formation of thiol-ester linkage between the carboxyl group of the fatty acid and SH group of coenzyme-A (CoA-SH; Eq. 10.20). The reaction is catalysed by **acyl-CoA synthetase**, also called fatty acid thiokinase.

$$R-\overset{\overset{O}{\|}}{C}-O^- + ATP + CoA-SH \rightarrow R-\overset{\overset{O}{\|}}{C}-SCoA + AMP + PP_i \quad \ldots(10.20)$$

An identical reaction was discussed in Unit 8 as part of esterification of acids. It may be recalled that the above reaction proceeds in two steps. In the first step, the fatty acid and ATP react at the enzyme active site with the formation of enzyme-bound acyl-adenylate (also called acyl-AMP). In the next step, acyl-adenylate reacts with CoA-SH with the formation of acyl-CoA and AMP. The structure of acyl-adenylate is given in Unit 8, Section 8.5.1.

$$R-COO^- + ATP \rightarrow \underset{\text{acyl - adenylate}}{R-CO-AMP} + PP_i$$

$$R-CO-AMP + CoA-SH \rightarrow R-CO-S-CoA + AMP$$

As mentioned in Unit 8, the equilibrium constant of the above reaction is close to unity, because the $\Delta G^{o\prime}$ values for the hydrolysis of ATP ($\rightarrow$ AMP + PP_i) and of acyl-CoA ($\rightarrow$ acylate + CoA- SH) are approximately equal (Unit 8, Table 8.1). The reaction is driven to completion by the removal of pyrophosphate ion (PP_i) which is hydrolysed to two phosphate ions in the presence of **pyrophosphatase.** Thus, the total reaction can be represented as:

$$R-COO^- + ATP + CoA-SH + H_2O \xrightarrow{\substack{\text{acyl - CoA - synthetase +} \\ \text{pyrophosphatase}}} R-CO-SCoA + AMP + 2P_i \quad \ldots(10.21)$$

In this reaction, two high energy pyrophosphate linkages have been broken and only one high energy thiol-ester linkage established. The extra energy is utilised to drive the reaction to completion in the desired direction. As mentioned earlier, this is a recurring feature of biosynthetic processes, i.e., "expenditure" of extra energy to drive the endergonic reaction to completion (also see gluconeogenesis, Unit 9).

10.4.2 Oxidative Degradation of Acyl-CoA

Acyl-CoA undergoes a sequence of four reactions, namely an oxidation, hydration (i.e., addition of a water molecule), another oxidation and a thiolytic cleavage. As a result of these reactions, it loses a two-carbon fragment in the form of acetyl-CoA. The residual acyl-CoA, which is now shorter by two carbon atoms, undergoes the same sequence of reactions and this is repeated till the entire long chain fatty acyl-CoA has been broken down to several molecules of acetyl-CoA.

The first oxidation reaction is catalysed by an acyl-CoA dehydrogenase with FAD as the hydrogen acceptor.

$$R-CH_2-CH_2-CO-SCoA + FAD \longrightarrow \underset{\substack{\text{trans-}\Delta^2\text{-enoyl-CoA}}}{R-\overset{\overset{H}{|}}{C}=\overset{\overset{H}{|}}{C}-CO-SCoA} + FADH_2 \quad \ldots(10.22)$$

The resulting trans-2,3-unsaturated enoyl-CoA adds a molecule of water in the presence of **enoyl-CoA hydratase.** The addition of water is stereospecific giving rise to L-3-hydroxyacyl-CoA, which is oxidised in the presence of L-3-hydroxy-acyl-CoA dehydrogenase with NAD^+ as the coenzyme.

$$R-CH=CH-CO-SCoA + H_2O \longrightarrow \underset{\text{L-3-hydroxyacyl-CoA}}{R-\overset{\overset{OH}{|}}{\underset{\underset{H}{|}}{C}}-CH_2-CO-SCoA} \quad ...(10.23)$$

$$R-CHOH-CH_2-CO-SCoA + NAD^+ \longrightarrow \underset{\beta\text{-ketoacyl-CoA}}{R-\overset{\overset{O}{||}}{C}-CH_2-CO-SCoA} + NADH + H^+ \quad ...(10.24)$$

In the final reaction of this series, β-ketoacyl-CoA reacts with CoA-SH in the presence of β-ketothiolase (also called thiolase) giving rise to acetyl-CoA and another acyl-CoA which is two carbons shorter than the starting one.

$$R-CO-CH_2-CO-SCoA + CoA-SH \rightarrow R-CO-SCoA + CH_3-CO-SCoA \quad ...(10.25)$$

The latter can again undergo the reactions similar to those of Eq. 10.22 to 10.25 and the process is repeated till the entire aliphatic chain is broken down to several molecules of acetyl-CoA. Note that as a consequence of reactions of Eq. 10.22 to 10.24, the β-methylene group of acyl-CoA is oxidised to a keto group. Therefore, the above pathway is referred to as the **β-oxidation pathway.** A summary of the reactions of this pathway is given in Fig. 10.4.

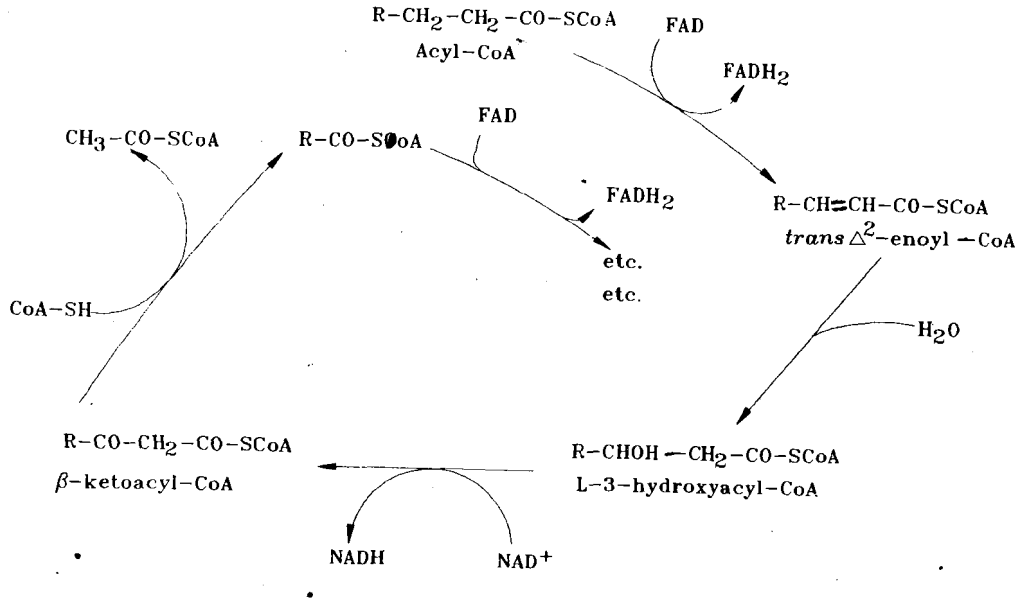


Fig. 10.4 : Summary of the reactions of β-oxidation pathway of fatty acids

Similarity of the reactions of Eq. 10.22 to 10.24 to the last three reactions of TCA cycle, namely succinate → fumarate → malate → oxaloacetate, is remarkable. It appears that similar reactions are utilised by nature in different metabolic pathways when the overall required chemical transformations are identical.

As a consequence of the reactions of Eq. 10.22 to 10.25, the acyl-CoA molecule loses a two-carbon fragment at a time. It is noteworthy that most of the fatty acids present in oils and fats have an even number of carbon atoms. Thus, the entire carbon chain is

broken down to acetyl-CoA molecules. Fatty acids with odd number of carbon atoms are less commonly found. In their case, a three-carbon fragment is left behind in the form of propionyl-CoA. The latter is carboxylated to succinyl-CoA which then enters the TCA cycle.

10.4.3 Energetics of the Oxidation of Fatty Acids

The oxidation of fatty acids yields large amount of ATP. Let us understand this by taking the example of a common fatty acid found in many oils and fats, namely palmitic acid. Its molecular formula is $C_{16}H_{32}O_2$ (i.e., $C_{15}H_{31}$-COOH). Two high-energy pyrophosphate linkages are utilised to convert it into its active form, namely, palmitoyl-CoA or $C_{15}H_{31}$-CO-SCoA. Since a two-carbon atom fragment is removed in each cycle of β-oxidation pathway, seven such cycles will be required for the complete breakdown of palmitoyl-CoA giving rise to eight molecules of acetyl-CoA. The stoichiometry of the β-oxidation breakdown of palmitoyl-CoA can thus be written as shown below leaving out H^+ and water molecules.

$$C_{15}H_{31} - CO - SCoA + 7\,FAD + 7NAD^+ + 7CoA - SH \longrightarrow 8CH_3 - CO - SCoA + 7FADH_2 + 7\,NADH \quad ...(10.26)$$

No useful energy is captured directly during the reactions of β-oxidation pathway. The energy transduction takes place only on the oxidation of acetyl-CoA and of the reduced coenzymes. It may be recalled from Section 10.3.3 that complete oxidation of the acetyl moiety of one acetyl-CoA molecule results in the net synthesis of twelve molecules of ATP. In addition, two molecules of ATP are synthesised on the oxidation of each $FADH_2$ molecule and three molecules are obtained on the oxidation of each NADH molecule. Thus, the total ATP synthesis from the products of the reaction is found to be equal to $(12 \times 8) + (7 \times 2) + (7 \times 3) = 131$ molecules for each molecule of palmitoyl-CoA. Since two energy-rich linkages were "spent" for the formation of palmitoyl-CoA from palmitate, net ATP synthesis will be 129 molecules per molecule of palmitate.

Standard free energy of oxidation of palmitic acid is reported to be equal to -9780 kJ mol^{-1}. The standard free energy for the synthesis of 129 ATP molecules is found to be equal to $129 \times (+\ 30.5) = 3935$ kJ mol^{-1}. Thus, the efficiency of energy transduction based on the **standard free energies** is $3935/9780 = 0.402$ or approximately 40%, which is similar to that of glycolysis, TCA cycle and oxidative phosphorylation.

In terms of the number of ATPs produced per carbon atom, it comes to 38/6 or ≈ 6 ATPs per carbon atom from glucose and 129/16 or ≈ 8 ATPs per carbon atom for palmitate. The chemical reason for this difference is that most of the carbons in palmitate are in the completely reduced state whereas glucose is partially oxidised, with six oxygens in the molecule.

It is interesting to compare the energetics of metabolic oxidation of glucose and palmitic acid **on mass basis.** One mole glucose (molar mass 180), on complete oxidation leads to the synthesis of 38 moles ATP, i.e., 38/180 or 0.211 mole ATP per g glucose. On the other hand one mole palmitic acid, molar mass 288, on complete oxidation causes the synthesis of 129 moles ATP corresponding to 129/288 = 0.448 mole ATP per g palmitic acid. Note that on mass basis fatty acid oxidation yields more than twice as many ATP molecules as are formed on the oxidation of carbohydrates. This is also true of the standard free energy of oxidation (or combustion) of fats and carbohydrates (approximately 33.9 and 15.9 kJ g^{-1}, respectively).

Having studied the fatty acid catabolism, let us see how these are synthesised, but before that try to answer the following SAQ.

SAQ 4

You can use the data of Table 8.1 from Unit 8 for answering the following questions.

a) For the following reaction,

$$RCOO^- + ATP + CoA - SH \longrightarrow RCOSCoA + AMP + PP_i$$

show that the equilibrium constant is close to unity.

.....

.....

b) What will be the $\Delta G^{o'}$ value for the following reaction:

$$RCOO^- + ATP + CoA - SH + H_2O \longrightarrow RCO - SCoA + AMP + 2P_i$$

.....

.....

.....

.....

10.4.4 Biosynthesis of Fatty Acids

Fatty acid biosynthesis takes place in most living systems. In each case, the starting material is acetyl-CoA. The biosynthesis is not merely a reversal of the β-oxidation pathway, but some new reactions are involved. Further, the site of biosynthesis and organisation of the participating enzymes are different. Major differences between the degradative and biosynthetic pathways are listed below:

- Degradation of fatty acids takes place in the mitochondrial matrix and peroxisomes while their synthesis takes place in the cytosol.
- The enzymes of fatty acid degradation seem to exist independently, but those involved in fatty acid biosynthesis exist either as a strong multienzyme complex, e.g., in bacteria, or are joined together covalently in a single protein, e.g., in higher organisms. In each case, it is referred to as fatty acid synthase.

Acyl carrier protein carries all the intermediates during fatty acid synthesis.

- In the breakdown of fatty acids, the acyl group is always attached to SH group of coenzyme-A. In biosynthesis the acyl group remains attached to SH group of the prosthetic group of a specific protein, called **acyl carrier protein** (ACP), which is a constituent of the fatty acid synthase.
- In fatty acid synthesis chain elongation takes place by a sequential addition of two-carbon atom fragment derived from acetyl-CoA, corresponding to the removal of two-carbon fragments in the degradative pathway. However, in the synthetic pathway acetyl-CoA is first "activated" by carboxylation to malonyl-CoA at the expense of a high energy linkage of ATP. The condensation reaction between malonyl-CoA and acyl moiety of acyl-ACP is driven to completion by the loss of CO_2.
- In contrast to the use of FAD and NAD^+ as oxidants in fatty acid degradation, the only reductant employed in the biosynthetic pathway is NADPH.

As stated above, the biosynthesis of fatty acids is catalysed by fatty acid synthase, which may be a multienzyme complex or may carry all the activities on different parts of a single protein. In each case, the reaction steps and the constituent catalytic activities are identical. Also, the growing acyl moiety remains covalently attached to the SH group of the acyl carrier protein (ACP). This SH group is part of a phosphopantethiene moiety which is covalently attached to a serine residue of ACP. Note that the same moiety also forms part of the structure of coenzyme-A, as shown in Fig. 10.5.

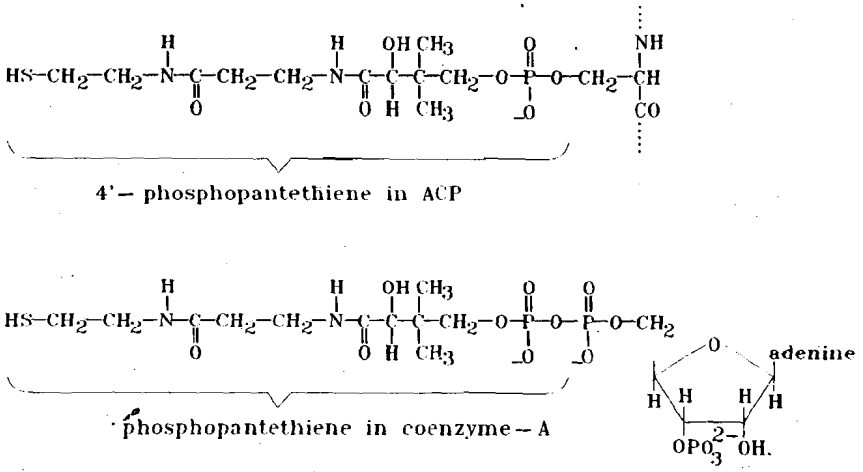


Fig. 10.5 : Phosphopantetheinyl moiety in ACP and coenzyme-A

Acetyl group of acetyl-CoA is first transferred to ACP. This reaction is catalysed by **acetyl transacylase.**

$$CH_3\text{-}CO\text{-}SCoA + HS\text{-}ACP \longrightarrow CH_3\text{-}CO\text{-}S\text{-}ACP + CoA\text{-}SH \quad ...(10.27)$$

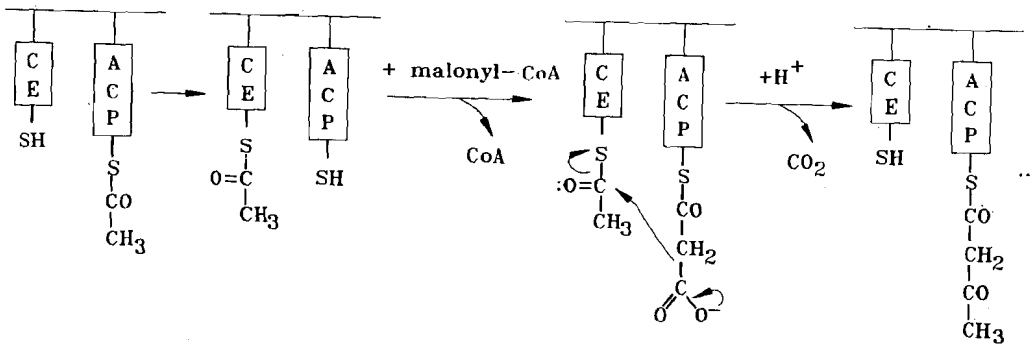
Another molecule of acetyl-CoA is "activated" by carboxylation. The reaction is catalysed by a biotin-enzyme, **acetyl-CoA carboxylase**, and requires the participation of ATP which is hydrolysed to ADP and P_i as shown below:

$$CH_3\text{-}CO\text{-}SCoA + ATP + HCO_3^- \longrightarrow CH_2(CO\text{-}SCoA)(COO^-) + ADP + P_i \quad ...(10.28)$$

malonyl-CoA

Note the similarity of this reaction to that of formation of oxaloacetate from pyruvate (Eq. 10.17). This is another example where chemically similar reactions are brought about by identical mechanisms even though they require different enzymes which are specific for different substrates.

The acetyl group of acetyl-ACP is transferred to the active site of condensing enzyme (CE in Eq. 10.29) and thus liberated SH group of ACP reacts with malonyl-CoA to give rise to malonyl-ACP. This is properly juxtaposed to the acetyl group attached to the condensing enzyme for the condensation reaction to take place and formation of acetoacetyl-ACP.



.... (10.29)

The condensation reaction is rendered irreversible by the loss of CO_2. Note the significant difference between this reaction and the corresponding reaction in the degradative pathway catalysed by **β-keto-thiolase** (Eq. 10.25).

Acetoacetyl-ACP undergoes successively a reduction, a dehydration and another reduction reaction catalysed by **β-ketoacyl-ACP reductase, 3-hydroxyacyl-ACP dehydratase** and **enoyl-ACP reductase**, respectively (Eq. 10.30). All these enzyme activities are constituents of fatty acid synthase.

$$CH_3\text{-}CO\text{-}CH_2\text{-}CO\text{-}SACP \xrightarrow[NADP^+]{NADPH} CH_3\text{-}CHOH\text{-}CH_2\text{-}CO\text{-}SACP \xrightarrow{-H_2O} CH_3\text{-}CH{=}CH\text{-}CO\text{-}SACP \xrightarrow[NADP^+]{NADPH} CH_3\text{-}CH_2\text{-}CH_2\text{-}CO\text{-}SACP \quad ...(10.30$$

As a result of the above reactions, the acyl chain of acetyl-ACP has grown by two carbon atoms to butyryl-ACP. The butyryl group is transferred to the condensing enzyme (as in the first step of Eq. 10.29) and another malonyl group is attached to ACP (as in the second step of Eq. 10.29). This is followed by the condensation (third step of Eq. 10.29) and the reactions of Eq. 10.30. The acyl chain has by now grown to six carbon atoms. This process goes on till palmitoyl-ACP ($C_{15}H_{31}$-CO-S-ACP) is formed. Palmitoyl moiety does not fulfill the specificity requirements of the condensing enzyme and is, therefore, not accepted by it. Instead, palmitoyl-ACP is a substrate for a hydrolytic enzyme, **thioesterase**, which is also one of the constituent enzymes of fatty acid synthase. Accordingly, it is hydrolysed releasing palmitate and making ACP available for starting synthesis of another fatty acyl chain.

$$CH_3-(CH_2)_{14}-CO-S-ACP + H_2O \rightarrow CH_3-(CH_2)_{14}-COO^- + HS-ACP \quad ...(10.31)$$

Further elongation of the aliphatic chain or the introduction of double bonds, where required, is brought about by other enzymes. These are not discussed here. Let us now compare the energetics of biosynthesis and degradation of fatty acids.

10.4.5 Comparison of Energetics of Biosynthesis and Degradation of Fatty Acids

Starting with acetyl-CoA and going through the reactions of Eq. 10.29 and 10.30 seven times each and finally hydrolysis (Eq. 10.31), we get

$$\text{acetyl-CoA} + 7\,\text{malonyl-CoA} + 14\,\text{NADPH} \longrightarrow \text{palmitate} + 7CO_2 + 14\,NADP^+ + 8\,\text{CoA} \quad ...(10.32)$$

Combining this with Eq. 10.28 on the formation of malonyl-CoA, we get the following reaction for the synthesis of palmitate (a C_{16} fatty acid) from eight molecules of acetyl-CoA.

$$8\,\text{acetyl-CoA} + 7\,ATP + 14\,NADPH \rightarrow \text{palmitate} + 14\,NADP^+ + 8\,\text{CoA} + 7\,ADP + 7\,P_i \quad ...(10.33)$$

For the β-oxidative breakdown of palmitate, you would recall that two high-energy pyrophosphate linkages are required to convert it into palmitoyl-CoA. Subsequently seven cycles of βeta-oxidation reactions produce eight moleules of acetyl-CoA with concomitant reduction of seven molecules each of NAD^+ and FAD. Thus, the total reaction may be written as,

$$\text{Palmitate} + ATP + 7\,NAD^+ + 7\,FAD + 8\,\text{CoA} \longrightarrow 8\,\text{acetyl-CoA} + 7\,NADH + 7FADH_2 + AMP + 2P_i \quad ...(10.34)$$

For comparing the energetics of reactions of Eq. 10.33 and 10.34, it is necessary to consider the **equivalents** of high energy linkages involved in the two cases. As explained earlier, each NADH or NADPH molecule is energetically equivalent to three ATP molecules or three high-energy linkages and each $FADH_2$ molecule is equivalent to two such linkages. Accordingly, the number of high-energy linkages "spent" in converting eight molecules of acetyl-CoA into one of palmitate is equal to $7 + (14 \times 3) = 49$ and the number of such linkage "gained" in the reaction of Eq. 10.34 is equal to $(7 \times 3) + (7 \times 2) - 2 = 33$. This number is different from that given in Sec. 10.4.3, because here we are considering the breakdown of palmitate to acetyl-CoA only and not its complete oxidation. Note that much more energy has to be "spent" or "pumped in" for performing biosynthetic work than can be "obtained" or "recovered" from the degradation of the synthesised molecule. The expenditure of extra energy helps to take the biosynthetic reaction to completion. It may be recalled that much more energy is spent in the synthesis of glucose from pyruvate than that recovered on the breakdown of glucose to pyruvate. These two examples help to illustrate a general principle. In all biosynthetic routes more energy is spent on the synthesis of a compound than what can be recovered by its metabolic breakdown. The extra energy helps to take the biosynthetic reaction to completion. This may partly explain the necessity of having different pathways for breakdown and biosynthesis.

SAQ 5

Fill in the blanks in the following:

i) Fatty acid biosynthesis takes place in ----------

ii) The cofactor of enzyme acetyl-CoA carboxylase is ----------

iii) The synthesis of palmitate via fatty acid synthesis cycle requires ----------molecules of malonyl-CoA.

iv) The formation of fatty acids from malonyl-CoA uses---------- as reductant.

10.5 SUMMARY

Let us summarise what we have discussed in this unit about the TCA cycle and metabolism of fatty acids.

Pyruvate formed on glycolysis or from alanine is converted into acetyl-CoA. This oxidative decarboxylation of pyruvate and condensation of the resultant acetyl moiety with thiol group of coenzyme-A is catalysed by an assembly of three enzymes known as pyruvate dehydrogenase complex. Acetyl-CoA is also formed by the breakdown of fatty acids. The acetyl moiety of acetyl-CoA is oxidised completely to carbon dioxide via a cyclic sequence of reactions, called Kreb's tricarboxylic acid (TCA) cycle or citric acid cycle. Acetyl moiety of acetyl-CoA is transferred to oxaloacetate to give rise to citrate. The latter undergoes a series to reactions in which two molecules of carbon dioxide are lost and oxaloacetate is regenerated. It then combines with another molecule of acetyl-CoA and the cycle goes on. TCA cycle occupies a central place in metabolism because it helps oxidise the metabolites formed from carbohydrates, amino acids and fats. In addition, some metabolites of the cycle serve as precursors in the synthesis of porphyrins and some amino acids. The concentrations of the metabolites of TCA cycle are not allowed to be depleted and are maintained at optimum levels by formation of oxaloacetate by carboxylation of pyruvate (anaplerosis).

The reduced coenzymes formed in the tricarboxylic acid cycle and earlier steps are reoxidised, and thereby regenerated via the electron transport chain with the net synthesis of ATP from ADP and phosphate ion. Calculations show that about 90% of the free energy transduction in carbohydrate catabolism (for the formation of ATP) takes place in the TCA cycle and electron transport chain.

Oils and fats are hydrolysed by various lipases to give glycerol and free fatty acids. Glycerol is metabolised via glycolysis. Each fatty acid is converted into the corresponding acyl-CoA which is transported from the cytoplasm to the mitochondrial matrix. Here through a sequence of reactions called β-oxidation, the entire aliphatic chain of acyl-CoA is broken down to several acetyl-CoA molecules. The acetyl group of the latter is then oxidised via TCA cycle. Biosynthesis of fatty acids utilises acetyl-CoA as the precursor and takes place in cytoplasm. It is catalysed by an assembly of several enzymes called fatty acid synthase. The latter contains a centrally located acyl carrier protein (ACP) surrounded by the enzymes which catalyse the various steps. Synthesis of palmitate via a sequence of reactions of fatty acid biosynthesis has been described in the unit. The unsaturated fatty acids and higher saturated fatty acids are all derived from palmitate. Much more energy is "spent" during biosynthesis of palmitate from eight molecules of acetyl-CoA than that which is "recovered" on breakdown of palmitate to eight acetyl-CoA molecules. This is generally true for any metabolism.

10.6 TERMINAL QUESTIONS

1) The following radioactively labelled compounds (position of label shown by an asterisk) are catabolised with a cell extract containing all the enzymes and cofactors of pyruvate dehydrogenase complex and TCA cycle. Predict the fate of the label (^{14}C), assuming only one turn of the TCA cycle.

(a) $CH_3^*-CO-COO^-$ (b) $CH_3-CO^*-COO^-$ (c) $CH_3-CO-COO^*$

(d) $CH_3^*-CO-SCoA$ (e) CH_3-CO^*-SCoA

2) Glucose was catabolised to carbon dioxide and water by minced pigeon breast muscle via glycolysis, TCA cycle and electron transport chain. The overall rate of metabolism was measured in terms of the rate of oxygen consumption. It was found that (i) addition of small amounts of oxaloacetate stimulated the rate of oxygen consumption and (ii) the increase in oxygen consumption was several times larger than that required to oxidise oxaloacetate to carbon dioxide and water. Explain (i) and (ii).

3) α-Ketoglutarate is required for the biosynthesis of several amino acids. From your study of this unit, write a sequence of known enzymatic reactions for a net synthesis of α-ketoglutarate without depleting the concentration of any metabolite of tricarboxylic acid cycle.

4) Compare the β-oxidation and biosynthesis of fatty acids with respect to the following aspects:

   a) intracellular location of the process

   b) nature of acyl group carrier

   c) nature of the oxidants and reductants employed and

   d) organisation of the participating enzymes

5) Given below are two statements. Explain whether they are true or false giving reasons.

   i) The methyl group of each acetyl-CoA molecule entering the TCA cycle is derived from the methyl group of pyruvate.

   ii) Malate cannot be converted to fumarate because the TCA cycle is unidirectional.

10.7 ANSWERS

Self Assessment Questions

1) (i) X (ii) √ (iii) √ (iv) X

2) (a) ii (b) i

3) (a) iv (b) iii

4) a) The reaction may be considered as the sum of two reactions:

$$ATP + H_2O \rightarrow AMP + PP_i;\ \Delta G^{o\prime} = -30.5\ kJ\ mol^{-1}$$

$$RCOO^- + CoA-SH \rightarrow R-CO-SCoA + H_2O;\ \Delta G^{o\prime} = -31.35\ kJ\ mol^{-1}$$

Thus the $\Delta G^{o\prime}$ is approximately equal to zero (0.8) and

$$\Delta G^{o\prime} = -2.303\,RT \log K_{eq}$$

value of $\log K'_{eq}$ will be 10^{-4} and therefore $K'_{eq} \approx$ unity.

b) The reaction may be considered to be the sum of three reactions:

$$ATP + H_2O \rightarrow AMP + PP_i;\ \Delta G^{o\prime} = -31.4\ kJ\ mol^{-1}$$

$$RCOO^- + CoA-SH \rightarrow R-CO-SCoA + H_2O;\ \Delta G^{o\prime} = 31.4\ kJ\ mol^{-1}$$

$$PP_i + H_2O \rightarrow 2\,Pi;\ \Delta G^{o\prime} = -33.0\ kJ\ mol^{-1}$$

5) (i) Cytosol (ii) biotin (iii) NADPH (iv) seven

Terminal Questions

1) a) Label is distributed in C-2 and C-3 of oxaloacetate.

b) Label is distributed in C-1 and C-4 of oxaloacetate.

c) Label is lost as CO_2 in the conversion of pyruvate to acetyl-CoA.

d) Same as in (a)

e) Same as in (b)

2) a) Oxaloacetate has a "catalytic" role in the TCA cycle, because it is regenerated at the end of the cycle and is used over and over again. Increasing the concentration of oxaloacetate increases the rate of formation of citrate, as observed with all enzymes at higher substrate concentrations. This increases the rate of reactions of tricarboxylic acid cycle, which is reflected in the enhanced rate of oxygen consumption.

b) Since oxaloacetate is regenerated, it is used over and over again for catabolism of more and more of glucose. Consequently, much larger amount of oxygen is consumed than that required for the oxidation of the added oxaloacetate only.

3)

4)

	β -Oxidation	Biosynthesis
a)	Mitochondrion	Cytoplasm
b)	Coenzyme-A	Acyl carrier protein
c)	FAD and NAD^+ (oxidants)	NADPH (reductant)
d)	Isolated as individual enzymes	Isolated as fatty acid synthase complex

5) i) False. The acetyl-CoA is also produced from β-oxidation of long chain fatty acids or from amino acid metabolism.

ii) False. Malate can easily be dehydrated to fumarate by reversal of fumarase reaction.