1

Main metabolism

Abstract: The main metabolic pathways, such as the glycolysis (EMP pathway), pentose phosphate pathway, the TCA cycle, the Entner Doudoroff pathway, as well as anaplerotic and gluconeogenetic pathways, are explained. The respiratory chain pathway is also explained in relation to energy generation. Amino acids and fatty acid synthetic pathways and various carbohydrate pathways are also explained.

Key words: Embden-Meyerhoff-Parnas pathway; glycolysis; pentose phosphate pathway; TCA cycle; respiratory chain; catabolism; anabolism; amino acid synthesis; fatty acid synthesis; carbohydrate metabolism.

1.1 Introduction

Metabolism describes the overall chemical or enzymatic reactions that occur in living organisms, which assimilate nutrients with high enthalpy and low entropy and gain free energy during the process of breakdown of nutrients into low enthalpy and high entropy substances, and thus keep the cells alive. All living organisms are in this irreversible state, and if this does not occur, the metabolic processes in the organism become in equilibrium, and the organism can no longer remain alive.

Let us define the **metabolite** as the substrate, intermediate, or product of each metabolic reaction in the cell, and let the **metabolic pathway** be a series of metabolic or enzymatic reactions from the specific substrate to the specific product. Typical central metabolic pathways are shown in Figure 1.1. When studying metabolic regulation or how cell metabolism is regulated, it is important to understand 'catabolism' and 'anabolism'.

Published by Woodhead Publishing Limited, 2013

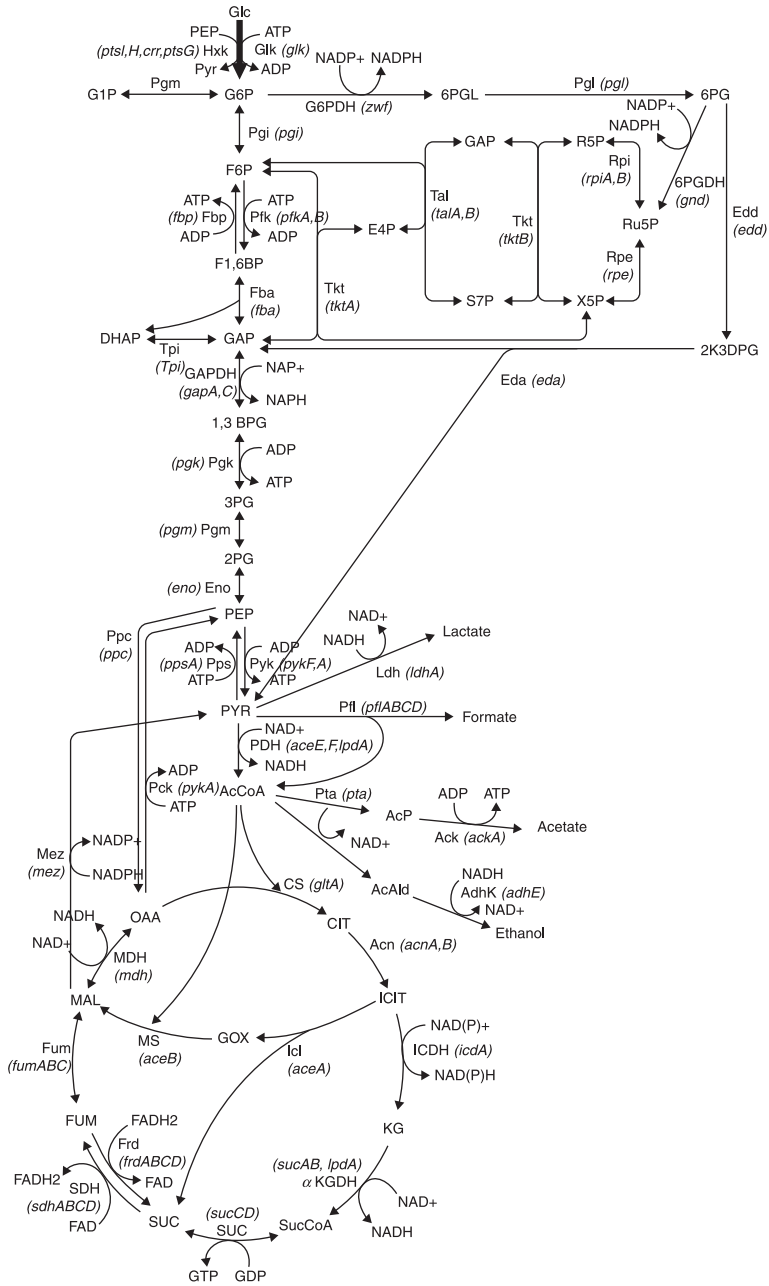


Figure 1.1 **Main metabolic pathways**

Published by Woodhead Publishing Limited, 2013

C**atabolism** is defined as all chemical or enzymatic reactions involved in the breakdown of organic or inorganic materials such as proteins, sugars, fatty acids, etc. in order to obtain energy. **Anabolism** is defined as biosynthetic reactions that lead to the building of cell materials such as proteins, DNA, RNA, lipids, etc. from small molecules, such as pyruvate, produced along the main metabolic pathways using the energy obtained by the process of catabolism.

Briefly, the cell generates energy as ATP (adenosine triphosphate), typically along the glycolysis pathway, from glucose to low molecules such as pyruvate (Figure 1.2). Moreover, the reducing equivalents, such as NADH and $FADH_2$, produced at the glycolysis and TCA (tri carboxylic acid) cycle pathways are oxidized in the respiratory chain, where ATP is produced via the oxidative phosphorylation process. Namely, these energy generating processes are termed **catabolism**. Cell constituents, such as proteins, cell membranes, etc. are formed from their precursor metabolites, such as 3-phosphoglycerate (3PG), phosphoenol-pyruvate (PEP), and pyruvate (PYR) etc., by using the ATP produced by the process of catabolism. This is the **anabolic process.**

1.2 Energy generation in the cell

The source of energy in a cell is ATP, which plays an essential role in a living organism. As shown in Figure 1.3, ATP contains high energy bonds, and if only one phosphate group of ATP is hydrolyzed, the free energy of this hydrolysis is 31 kJ (7.3 kcal), such that:

$$ATP + H_2O \rightarrow ADP + P_i \quad \Delta G = -7.3 \text{ kcal/mol} \tag{1.1}$$

under normal conditions with a pH at 7.0 and the temperature at 25°C. The active ATP often forms complex compounds with Mg^{2+} ions, which can result in a shift of the equilibrium. As stated above, during catabolism, the living organism has to generate ATP from ADP and inorganic phosphate P_i (HPO_3^{2+}) by reversing the above reaction, which is then stored by the cell to keep it alive. Note that ATP analogs, such as guanosine 5′-triphosphate (GTP), uridine 5′-triphosphate (UTP), and cytidine 5′-triphosphate (CTP), are also high energy compounds also comparable to ATP.

An enzyme that uses ATP as a substrate is called **transferase**, and an enzyme that transfers γ-phosphate of ATP to the substrate is called **kinase**:

$$S + ATP \rightarrow S\text{-phosphate} + ADP \tag{1.2}$$

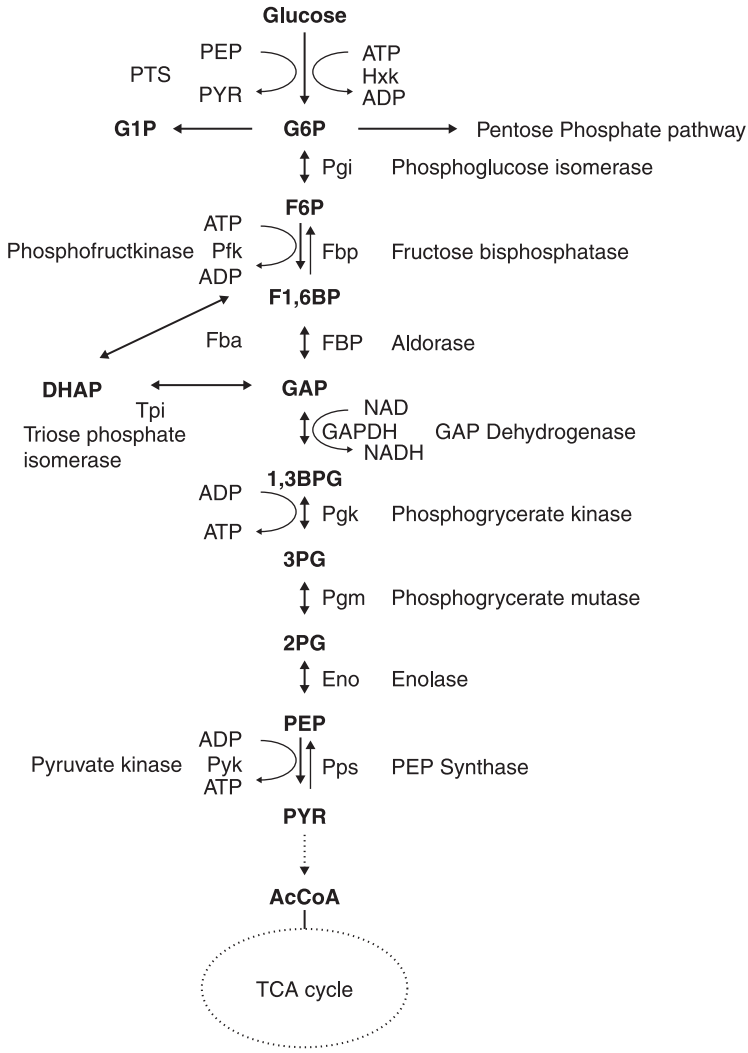


Figure 1.2 **Metabolic pathways of glycolysis**

where hexokinase (Hxk) and phosphofructokinase (Pfk), etc. belong to this category. Other kinases, such as phosphoglycerate kinase (Pgk), pyruvate kinase (Pyk), and acetate kinase (Ack), are those generating ATP. It may be useful to evaluate the energy charge as (Atkinson, 1968):

$$\text{Energy Charge} = \frac{[ATP]+0.5[ADP]+0.0[AMP]}{[ATP]+[ADP]+[AMP]} \tag{1.3}$$

Published by Woodhead Publishing Limited, 2013

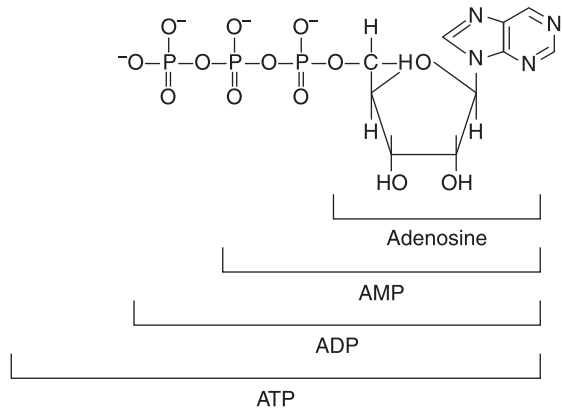


Figure 1.3 Structure of ATP

where [ ] denotes the concentration.

In relation to ATP, the **reducing equivalents**, such as NADH, $FADH_2$, and NADPH, also play important roles in cell metabolism. Their chemical structures are shown in Figure 1.4, where NADH is utilized for energy generation by oxidative phosphorylation along the respiratory chain, and thus related to catabolism, while NADPH is the reducing power for amino acid synthesis or fatty acid formation, etc., and thus related to anabolism.

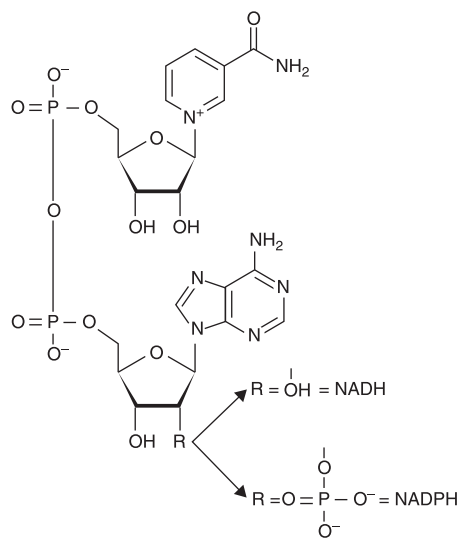


Figure 1.4 Structures of NADH and NADPH

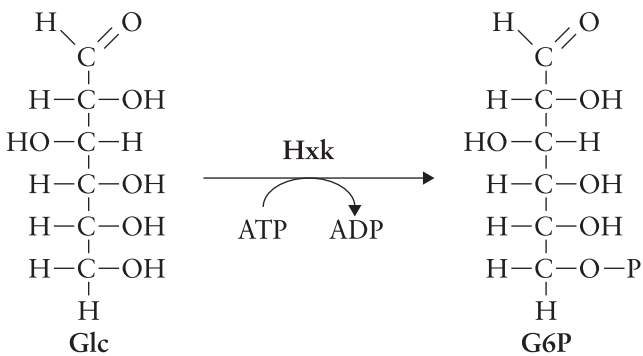
NADH and NADPH can be inter-convertible by transhydrogenase in some bacteria, where Udh converts NADPH to NADH, while Pnt converts NADH to NADPH in *Escherichia coli*, which will be explained in more detail in relation to metabolism in later chapters. Although there are many biochemistry textbooks such as Horton et al. (1996), Lehninger et al. (1993), Stryer (1995), Voet and Voet (1995) etc., let us consider briefly the metabolic pathways one by one from the next section.

1.3 Carbohydrate metabolism

The typical carbohydrate for bacteria is glucose, which is converted to pyruvic acid (PYR) mainly via three different pathways such as the Embden-Meyerhof-Parnas (EMP) pathway, the pentose phosphate (PP) pathway (or hexose monophosphate: HMP) pathway, and the Entner-Doudoroff (ED) pathway. The EMP pathway is often referred to as glycolysis, but the PP and ED pathways can also be called glycolysis in some cases.

1.3.1 EMP pathway

The first step in glucose breakdown is the phosphorylation step that requires 1 mole of ATP catalyzed by hexokinase (Hxk) (ATP: D-hexose phosphotransferase, EC 2.7.1.1) to produce glucose 6-phosphate (G6P):



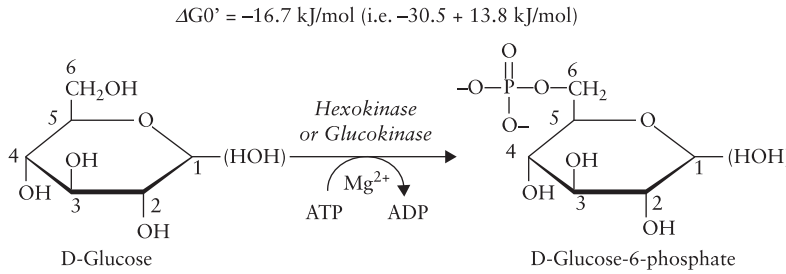
(1.4)

Note that enzymatic function relates to the catalytic specificity of an enzyme, which is described by its Enzyme Commission (EC) number

Published by Woodhead Publishing Limited, 2013

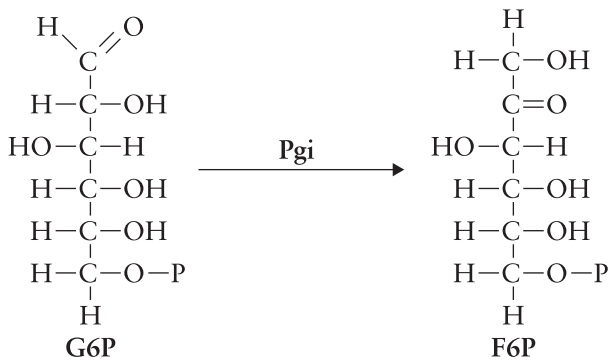
(Webb, 1992). Each enzyme-catalized reaction is classified based on a four-digit EC number, where the first number specifies the class of enzymes (1, oxidoreductases; 2, transferases; 3, hydrolases; 4, lyases; 5, isomerases; and 6, ligases). Subsequent digits provide additional function.

The detailed reaction scheme may be expressed as:



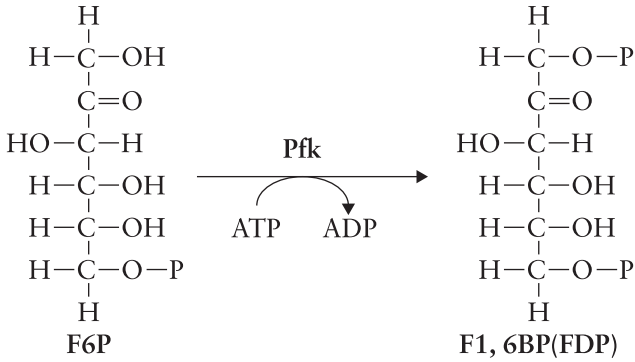
Another phosphorylation system (phosphotransferase system, PTS) using phosphoenol pyruvate (PEP) will be explained later in this and later chapters.

The second step is isomerization of G6P to fructose 6-phosphate (F6P) by the enzyme glucose phosphate isomerase, Pgi (D-glucose-6-phosphate-isomerase, EC 5.3.1.9), where this pathway is highly reversible:



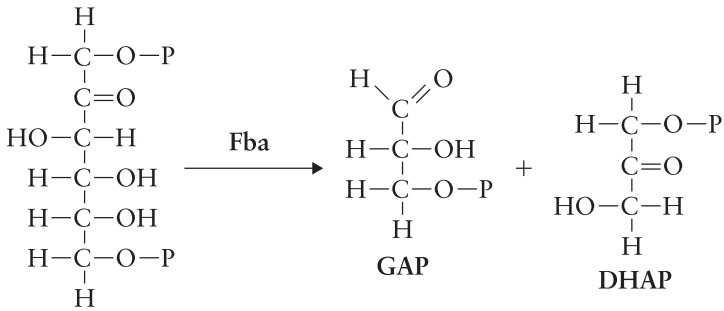
(1.5)

The third step or the second phosphorylation step requires additional ATP for phosphorylation to produce fructose 1,6-bisphosphate (F16BP or FDP) from F6P. This reaction is catalyzed by the important enzyme, phosphofructokinase (Pfk) (ATP: D-fructose-6-phosphate 1 phosphotransferase, EC 2.7.1.11), which regulates the flow through the EMP pathway, and sometimes becomes a rate-limiting factor of the EMP pathway. It has been shown that Pfk is allosterically inhibited by PEP:



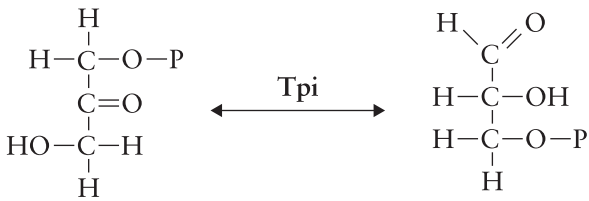
F6P F1, 6BP(FDP) (1.6)

The next step is the cleavage of 1,6-diphosphate (F16BP or FDP) to 2 moles of triose phosphate. The enzyme that catalyzes this reaction is fructose diphosphate aldolase (Fba) (fructose-1,6-bisphosphate: D-glyceraldehyde-3-phosphate-lyase, EC 4.1.2.13):



F1, 6BP GAP DHAP (1.7)

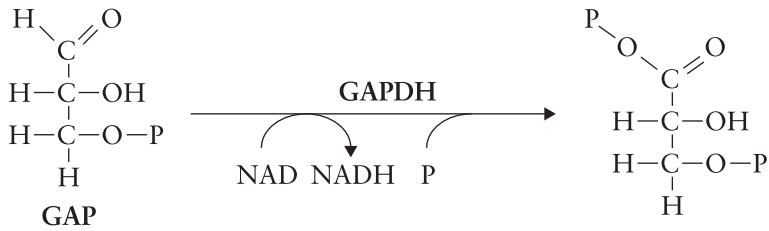
The cleavage of FDP to 2 moles of triose phosphate, such as glyceraldehyde 3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP), can be interchangeable with the reversible triose phosphate isomerase (Tpi) (D-glyceraldehyde-3-phosphate ketol-isomerase, EC 5.3.1.1). The equilibrium occurs from DHAP toward GAP, assuming the EMP pathway functions properly:



DHAP GAP (1.8)

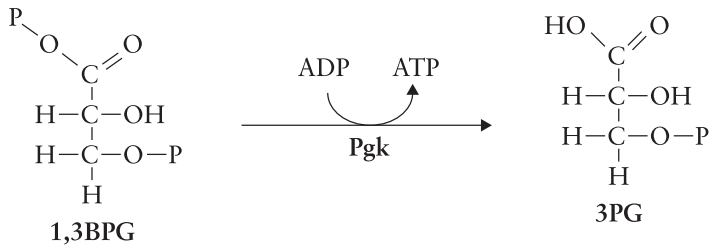
Published by Woodhead Publishing Limited, 2013

Step 6 is a combined oxidation and phosphorylation step, which is catalyzed by glyceraldehyde-phosphate dehydrogenase GAPDH (D-glyceraldehyde-3-phosphate: NAD oxidoreductase, EC 1.2.1.12), and produces NADH and the high-energy component 1,3-bisphospho-D-glycerate (1,3BPG):



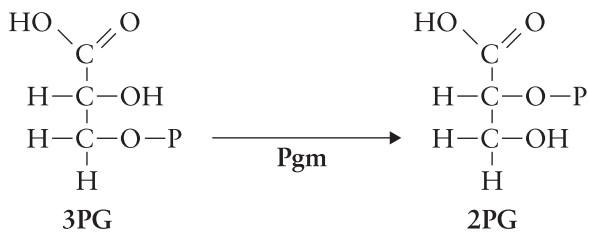
(1.9)

In step 7, the high-energy compound 1,3 BPG releases 1 phosphate group as 1 mole of ATP by the reaction catalyzed by phosphoglycerate kinase (Pgk) (ATP: 3-phospho-D-glycerate 1-phospho-transferase, EC 2.7.2.3) to produce 3-phosphoglycerate (3 PG):



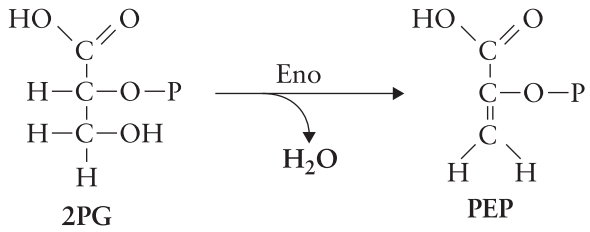
(1.10)

The conversion of 3 PG to 2-phosphoglycerate (2 PG) is catalyzed by phosphoglycerate mutase (Pgm) (2,3-diphospho-D-glycerate: 2 phospho-D-glycerate phosphostransferase, EC 2.7.5.3). In this reaction, the phosphate group is transferred from the third position to the second position:



(1.11)

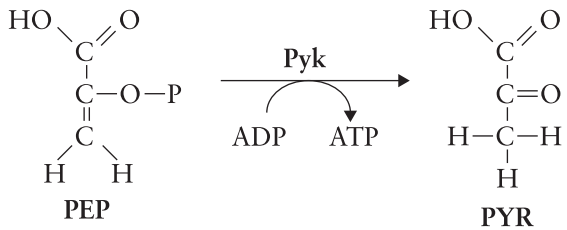
The next reaction step is catalyzed by enolase (Eno) (2-phospho-D-glycerate hydro-lyase, EC 4.2.1.11) to form phosphoenol pyruvate (PEP) (step 9):



(1.12)

This reaction is connected with an intracellular electron shift, often referred to as the intra-molecular oxidation-reduction reaction. By this reaction, PEP is more energy-rich as compared to 2 PG.

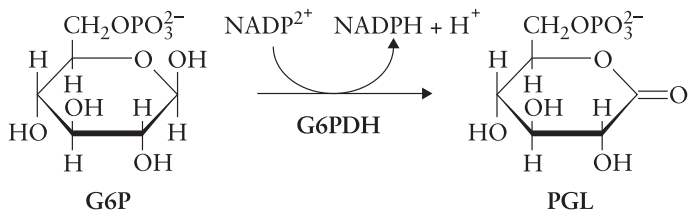
In the final step (step 10) of the EMP pathway, the reaction is catalyzed by pyruvate kinase (Pyk) (ATP: pyruvate phosphotransferase, EC 2.7.1.40) to produce pyruvate (PYR), and the phosphate group of PEP is transferred to ADP to produce ATP. Pyk is the second allosteric enzyme in the EMP pathway:



(1.13)

1.3.2 Pentose phosphate (PP) pathway or hexose monophosphate (HMP) pathway

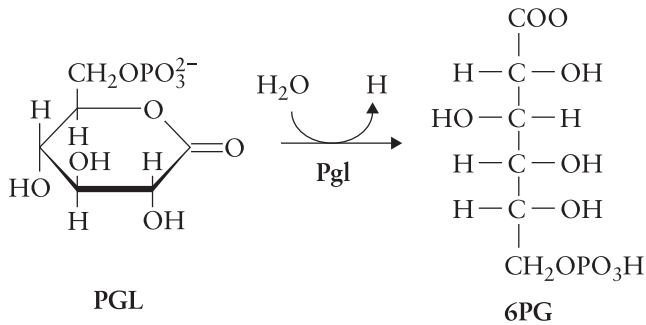
Figure 1.5 shows the overall pentose phosphate (PP) pathway, which connects to the EMP pathway at G6P, F6P, and GAP. In the PP pathway, G6P is oxidized by the $NADP^+$-linked G6P dehydrogenase (G6PDH) (*D*-glucose-6-phosphate: NADP oxidoreductase, EC 1.1.1.49) to produce D-glucono-δ-lactone 6-phosphate (PGL). G6PDH is an important regulatory enzyme, where it is inhibited by NADPH:



(1.14)

Published by Woodhead Publishing Limited, 2013

The product of the G6PDH reaction is almost immediately hydrolyzed to 6-phosphogluconate (6PG) by gluconolactonase (D-glucono-δ-lactone hydrolase, EC 3.1.1.17):



PGL 6PG (1.15)

A second $NADP^+$-linked oxidation produces D-ribulose 5-phosphate (Rib5P) from 6 PG this reaction is catalyzed by phosphogluconate dehydrogenase (6PGDH), where the C-1 atom of 6 PG is released as CO_2 to form Rib5P. 6PGDH is also an important regulatory enzyme, where it is inhibited by NADPH:

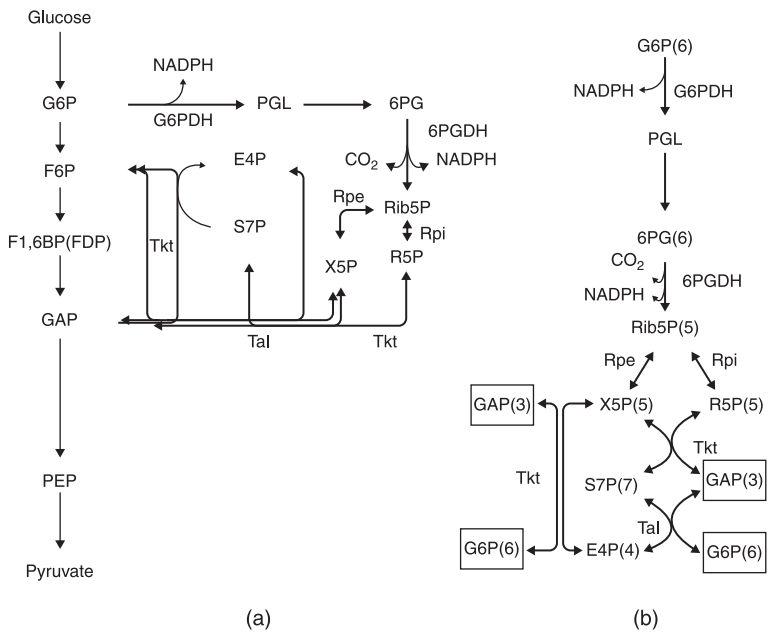
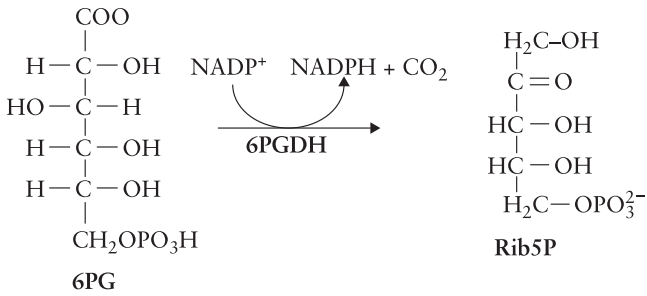
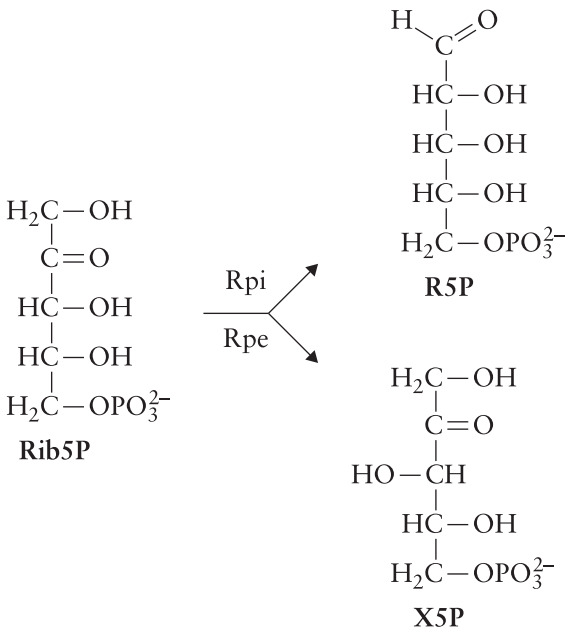


Figure 1.5 (a) Pentose phosphate pathway, and (b) the change in carbon numbers



(1.16)

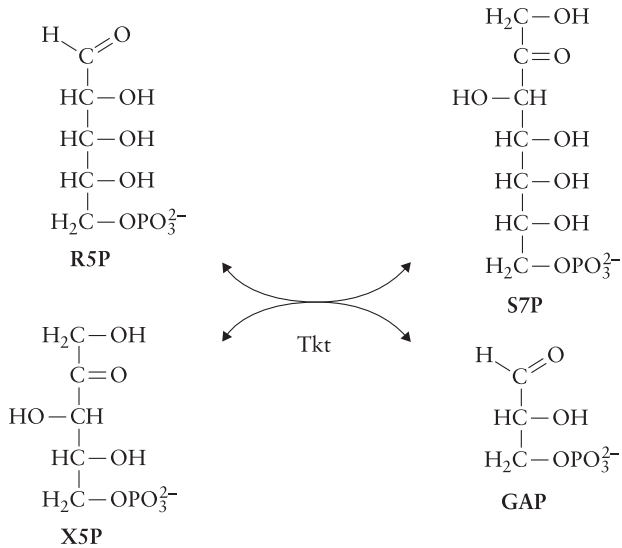
Rib5P is attacked partly by two different enzymes, such as ribose phosphate 3-epimerase (Rpe) (D-ribulose-5-phosphate 3-epimerase, EC 5.1.3.1), which converts Rib5P to xylulose 5-phosphate (X5P), and ribose 5-phosphate isomerase (Rpi) (D-ribose-5-phosphate ketol-isomerase, EC 5.3.1.6), which converts Rib5P to ribose 5-phosphate (R5P):



(1.17)

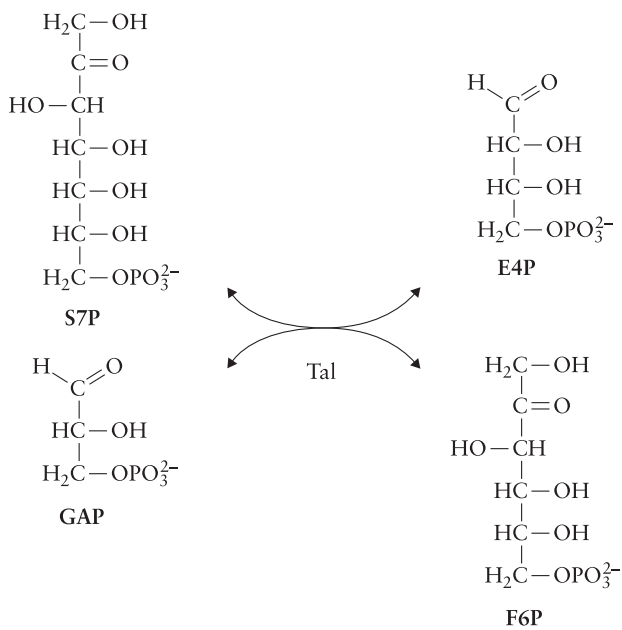
Both intermediates, such as X5P and R5P, are required for the cleavage reaction catalyzed by transketolase (sedoheptulose-7-phosphate: D-glyceraldehyde-3-phosphate glycolaldehyde transferase, EC 2.2.1.1), which yields GAP and sedoheptulose 7-phosphate (S7P):

Published by Woodhead Publishing Limited, 2013



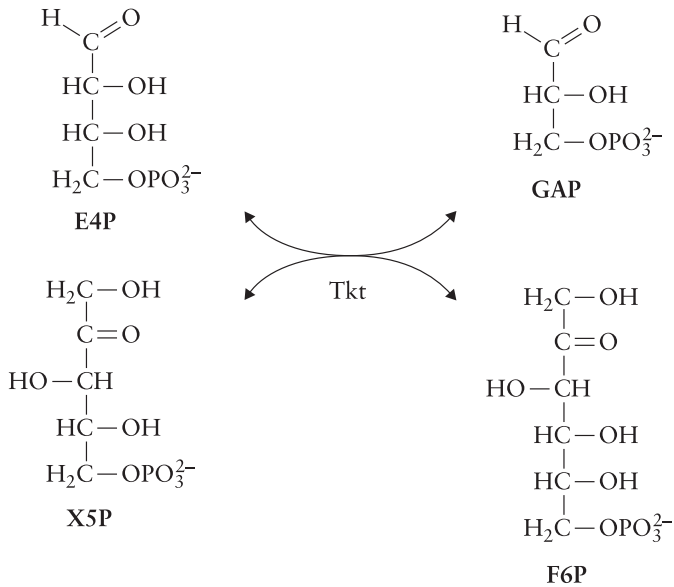
(1.18)

The second cleavage reaction cleaves both intermediates from the transketolase reaction to produce F6P and erythrose 4-phosphate (E4P), where this reaction is catalyzed by transaldorase (D-glyceraldehyde-3-phosphate dehydroxy acetone transferase, EC 2.2.1.2). Note that E4P and R5P are the important precursors for purine, pyrimidine, and aromatic amino acids:



(1.19)

The third cleavage reaction is carried out by the same transketolase as the first stage reaction, and cleaves E4P and X5P to form GAP and F6P:

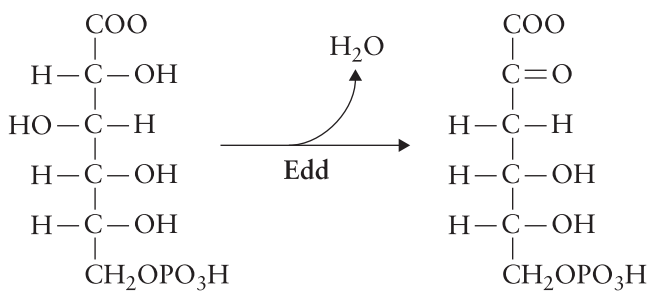


(1.20)

Note that the pathway reaction from G6P to Rib5P is unidirectional and is known as the **oxidative PP pathway**, while the other reactions in the PP pathway are reversible, so it is called the **non-oxidative PP pathway**.

1.3.3 Entner-Doudoroff pathway

As shown in Figure 1.6, the Entner-Doudoroff (ED) pathway connects to the PP pathway at 6 PG, where 6 PG is converted to 2-keto-3-deoxy-6-phosphogluconate (KDPG) by dehydration reaction catalyzed by phospho-gluconate dehydratase (Edd) (6-phosphogluconate hydro-lyase, EC 4.2.1.12):



(1.21)

Published by Woodhead Publishing Limited, 2013

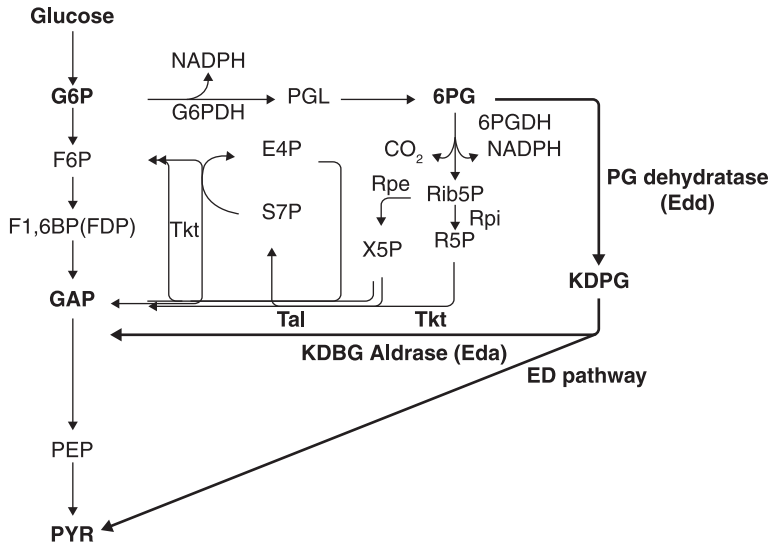
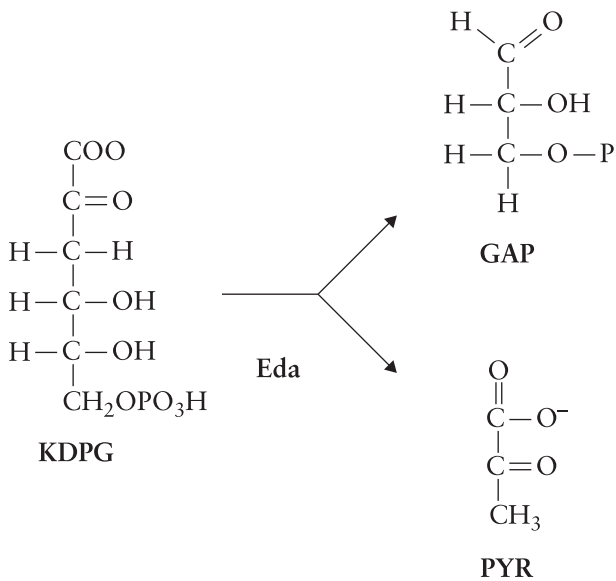


Figure 1.6 Entner Doudoroff pathway

The next step is the cleavage of KDPG to GAP and PYR by phospho-2-keto-3-deoxy-gluconate aldorase (Eda) (6-phospho-2-keto-3-deoxy-D-gluconate D-glyceraldehyde-3-phosphate lyase, EC 4.1.2.14):



(1.22)

Although the ED pathway is active in such microorganisms as *Zymomonas mobilis*, this may be induced in other bacteria such as *E. coli*, depending on the genetic and culture conditions, as will be explained later in this book.

1.3.4 PDH and TCA cycle

As shown in Figure 1.7, the terminal product of EMP or ED pathways is PYR, where it is converted to a two carbon acid derivative, such as acetyl CoA (AcCoA), by releasing CO_2 from the first carbon of PYR by the pyruvate dehydrogenase (PDHc) reaction, where this is a multi-enzyme complex consisting of three different enzymes as well as the cofactors, thiamine pyrophosphate (TPP), lipoic acid, and NAD^+.

The three enzymes are pyruvate dehydrogenase (pyruvate: lipoate oxidoreductase, EC 1.2.4.1), lipoate acetyltransferase (acetyl-CoA:

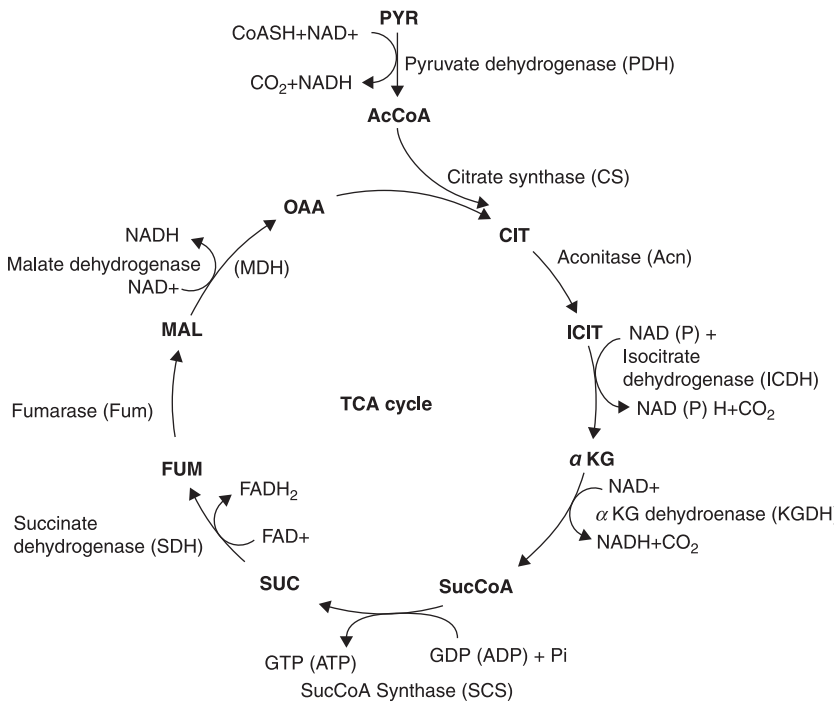
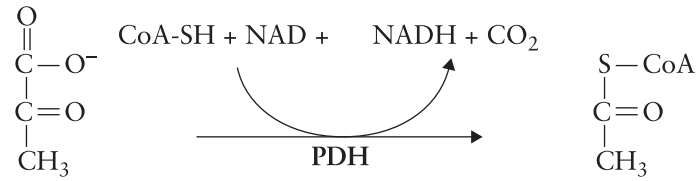


Figure 1.7 PDH and TCA cycle

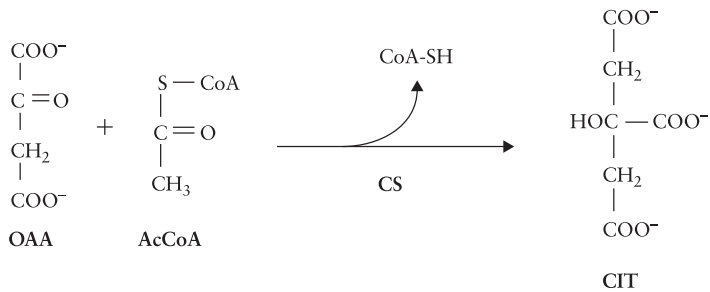
Published by Woodhead Publishing Limited, 2013

dihydrolipoate S-acetyltransferase, EC 2.3.1.12), and lipoamid dehydrogenase (reduced-NAD: lipoamid oxidoreductase, EC 1.6.4.3):



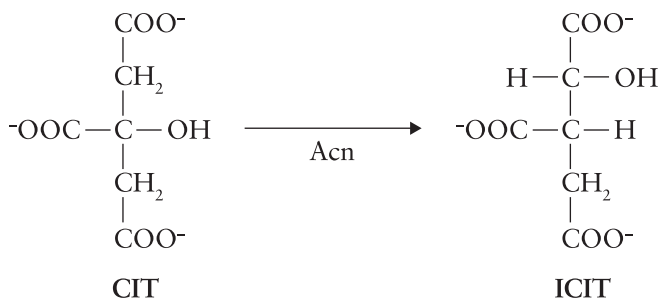
(1.23)

The acetyl CoA thus produced from PYR goes into a series of reactions called either the tricarboxylic acid (TCA) cycle, the Krebs cycle, or the citric acid cycle (Figure 1.7). In the first step of the TCA cycle, AcCoA gives the acetyl group to the four carbon dicarboxylic acids such as oxaloacetate (OAA) to form a six-carbon tricarboxylic acid such as citric acid (CIT). This reaction is catalyzed by citrate synthase (CS) (citrate oxaloacetate-lyase, EC 4.1.3.7), where free CoA is generated, and this can be re-utilized in the formation of AcCoA. Note that the end products of the TCA cycle are NADH and CO_2, where NADH allosterically inhibits the activity of CS:



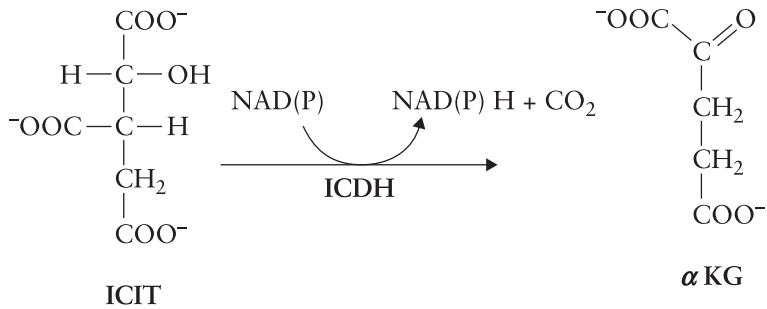
(1.24)

The next steps are the formation of *cis*-aconitate and then isocitrate by the enzyme aconitate hydratase or aconitase (Acn) (citrate (iso-citrate) hydro-lyase, EC 4.2.1.3):



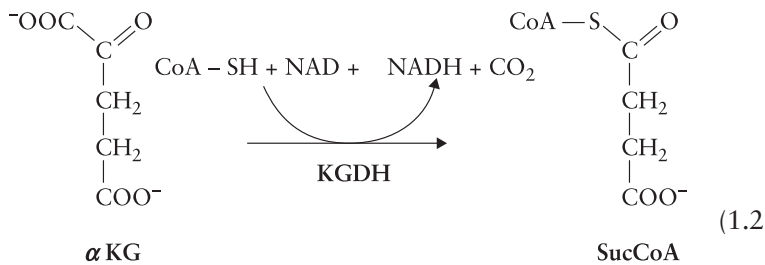
(1.25)

Isocitric acid (ICIT) is then converted to α-ketoglutaric acid (αKG) or 2-oxoglutarate (2KG) by the reaction catalyzed by isocitrate dehydrogenase (ICDH) (threo-D_s-isocitrate: NADP oxidoreductase, EC 1.1.1.42). NAD(P)H and CO_2 are formed through this reaction, where the microorganisms possess predominantly the $NADP^+$-specific ICDH, whereas fungi and yeasts possess the NAD^+-specific ICDH (Doelle, 1975):



(1.26)

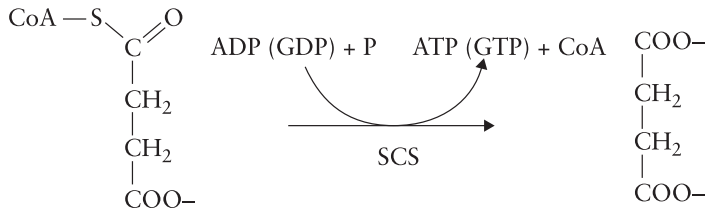
The next reaction step is the conversion of αKG to succinyl CoA (SucCoA) by the 2-oxoglutarate dehydrogenase complex (KGDH) (2-oxoglutarate lipoate oxidoreductase, EC 1.2.4.2), a multi-enzyme complex system, similar to PDH. The KGDH complex requires the participation of thiamine pyrophosphate (TPP), α-lipoic acid, CoA, NAD^+, and Mg^{2+}, to produce CO_2 and NADH:



(1.27)

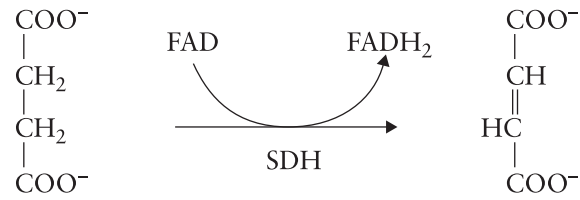
SucCoA is then converted to succinate (SUC) by succinyl-CoA synthetase (SCS) (succinate: CoA ligase, EC 6.2.1.5). Through this reaction step, CoA is released and ATP (GTP) is formed:

Published by Woodhead Publishing Limited, 2013



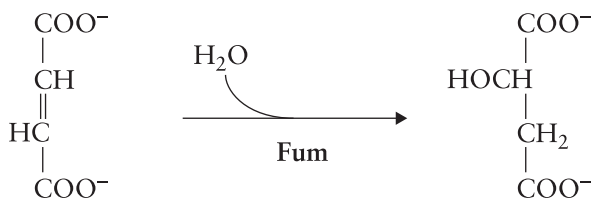
SucCoA **SUC** (1.28)

The next step is the dehydrogenation of SUC, where SUC is oxidized to fumarate (FUM) by succinate dehydrogenase (SDH) (succinate: oxidoreductase, EC 1.3.99.1). SDH is closely linked to the electron transport chain, and enters this system at the flavoprotein level, where $FADH_2$ is released by this reaction step:



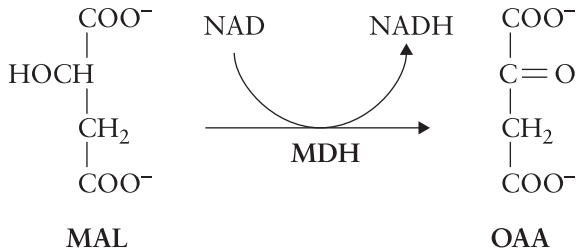
SUC FUM (1.29)

Fumarate thus formed is then hydrated at the double bond to form malic acid (MAL) by fumarase or fumarate hydratase (L-malate hydrolyase, EC 4.2.1.2):



FUM **MAL** (1.30)

The final reaction in the TCA cycle is the dehydrogenation of MAL to oxaloacetate (OAA) catalyzed by malate dehydrogenase (MDH) (L-malate: NAD oxidoreductase, EC 1.1.1.37), where NADH is produced in this reaction step:



(1.31)

1.3.5 Acetate metabolism

Microorganisms such as *E. coli* produce acetate from AcCoA by the so-called overflow metabolism, while ethanol is formed from PYR in yeast, etc. by a similar mechanism. There are two major acetate producing pathways, the phosphoacetyltransferase/acetate kinase (Pta-Ack) and pyruvate oxidase (Pox) pathways (Figure 1.8). The phosphoacetyltransferase (Pta) [EC 2.3.1.8] converts AcCoA and inorganic phosphate to acetyl phosphate (AceP) and CoA, while acetate kinase (Ack) [EC 2.7.2.1] converts AceP and ADP to acetate (Ace) and ATP in reversible reactions. Note that the cells may require ATP by the Pta-Ack pathway as the second energy source during high growth rates and anaerobic conditions, and that the Pta-Ack pathway may play an important role in the regulation of AceP, where AceP plays a role in metabolic regulation, as will be explained later in this book. The other acetate producing pathway is through pyruvate oxidase (Pox) [EC 1.2.2.2], a peripheral membrane protein that converts PYR, ubiquinone, and H_2O to acetate, ubiquinol, and CO_2, respectively. This pathway is usually induced at the early stationary phase, where acetyl-CoA synthetase (ACS) (acetate: CoA ligase, EC 6.2.1.1) is also activated to convert acetate to AcCoA at the stationary phase.

1.3.6 Anaplerotic pathways

There may exist a close interrelationship between catabolism for energy generation and anabolism for biosynthesis. Some of the intermediates in the main metabolic pathway are necessary as precursors for biosynthesis, as the cell can no longer survive without any of these. Thus the organism must have an ancillary system to take care of replenishment of these intermediates. The routes required for this replenishment are called **anaplerotic** routes (Doelle, 1975). Among the intermediates, OAA is often critical, since its concentration is relatively low due to its utilization by the CS reaction, while it is also a precursor for many amino acids such

Published by Woodhead Publishing Limited, 2013

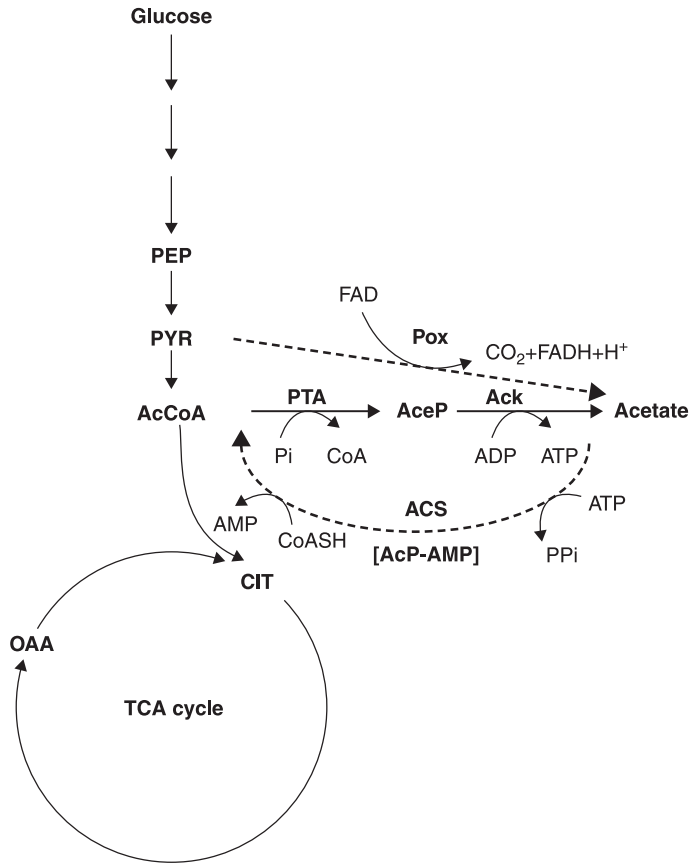
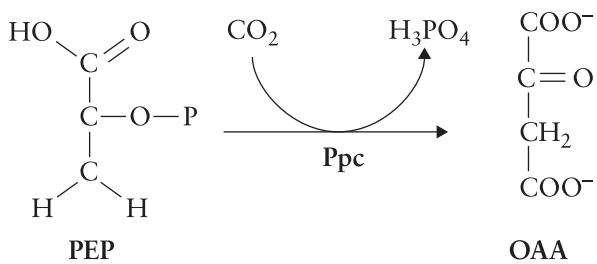


Figure 1.8 Acetate producing pathways

as aspartate and lysine, etc. There are, therefore, several anaplerotic pathways to prevent OAA shortage (Figure 1.9).

The typical anaplerotic pathway is a phosphoenol pyruvate carboxylase (Ppc) (orthophosphate: oxaloacetate carboxy-lyase, EC 4.1.1.31), which catalyzes the reaction of replenishing OAA from PEP as:



PEP OAA (1.32)

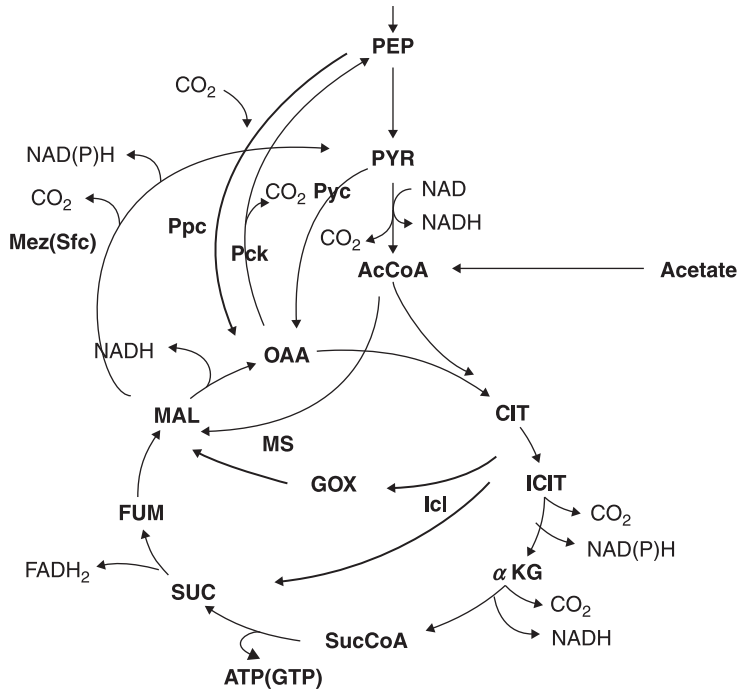
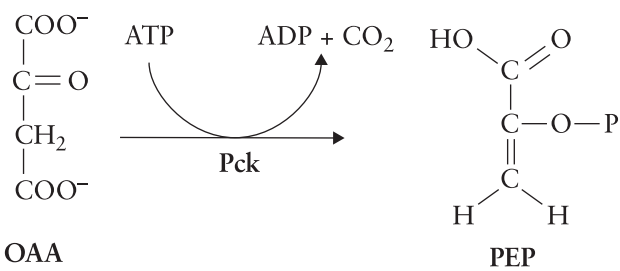


Figure 1.9 Anaplerotic pathways

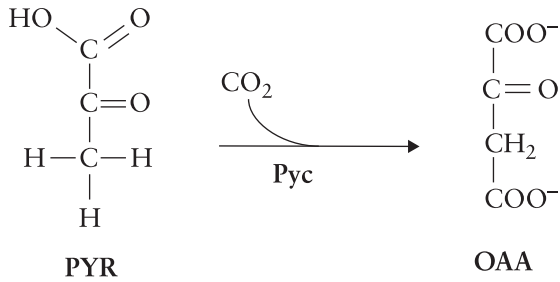
or the gluconeogenetic pathway, such as PEP carboxykinase (ATP: oxaloacetate carboxy-lyase, EC 4.1.1.49), which converts PEP to OAA and can be also utilized as the anaplerotic pathway by reversing the reaction:



(1.33)

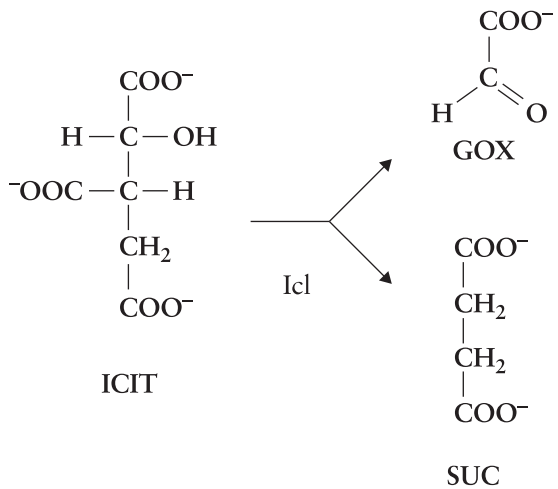
There is another pathway from PYR to OAA catalyzed by a biotin-dependent pyruvate carboxylase (EC 6.4.1.1); *E. coli* does not have this pathway, while *Corynebacteria*, etc. do have this pathway, i.e.:

Published by Woodhead Publishing Limited, 2013



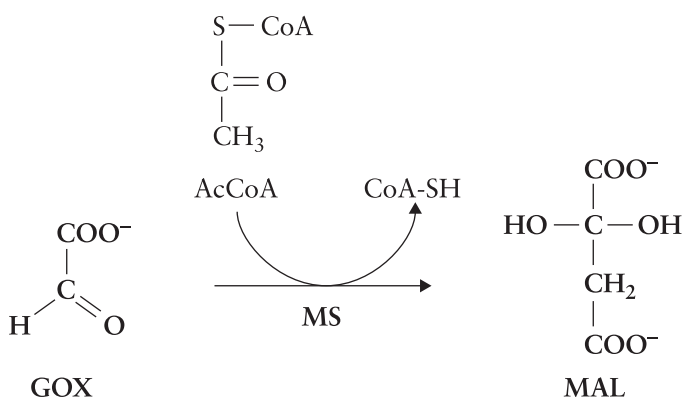
(1.34)

Another important anaplerotic pathway is the **glyoxylate pathway**, which consists of isocitrate lyase (Icl) (threo-D_s-isocitrate-glyoxylate-lyase, EC 4.1.3.1) and malate systhase (MS) (L-malate glyoxylate-lyase, EC 4.1.3.2). ICIT undergoes an aldo cleavage to SUC and glyoxylate (GOX) by Icl as:



(1.35)

The next step is the condensation of AcCoA with GOX to form MAL by MS:



(1.36)

This glyoxylate pathway has suggested the existence of a cyclic mechanism for replenishing C_4 acids from the TCA cycle for biosynthesis, and this forms the bypass of the TCA cycle and so forms the upper TCA cycle, and plays an important role in the metabolism of short-chain fatty acids (Doelle, 1975) as well as the gluconeogenetic pathway, and is sometimes called the glyoxylate cycle or glyoxylate shunt.

1.3.7 Gluconeogenesis

Acetate or fatty acid can be assimilated and metabolized via the TCA cycle and glyoxylate pathway when glucose is unavailable, and biosynthesis can be made via gluconeogenesis, where acetate is first converted to AcCoA by ACS (Figures 1.8 to 1.10). The MAL thus formed via either

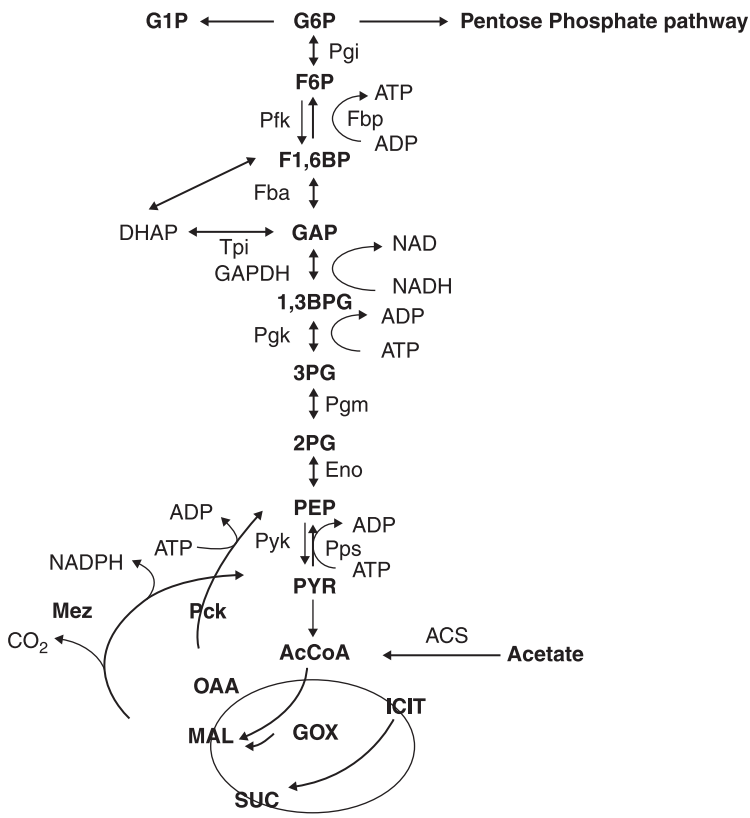
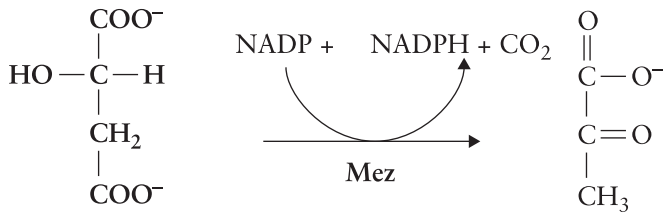


Figure 1.10 Gluconeogenetic pathways

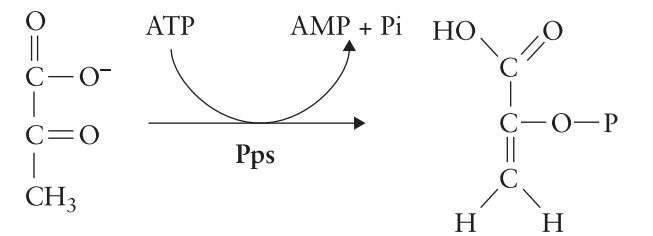
Published by Woodhead Publishing Limited, 2013

the TCA cycle or glyoxylate pathway is converted to PYR by malic enzyme (Mez) (L-malate: NADDP oxidoreductase, EC1.1.1.40), which catalyzes the oxidative carboxylation of OAA forming NADPH + H^+, such as:



MAL PYR (1.37)

The PYR thus formed is phosphorylated to form PEP by PEP synthase (Pps) (EC 2.7.9.2), requiring Mg^{2+}, K^+, and ATP, such that:



PYR PEP (1.38)

The OAA in the TCA cycle is converted to PEP by Pck, as mentioned above. Most of the EMP pathway reactions are reversible, but Pfk must be replaced by other enzymes, such as fructose bisphosphatase (Fbp) (EC 3.1.3.11), to convert FDP to F6P:

(1.39)

1.4 Respiratory chain pathways

Microbial cells can generate energy as ATP under a wide range of redox conditions. The reducing equivalents, such as NADH and $FADH_2$, are re-oxidized in the respiratory chain, where oxygen, nitrate, fumarate, and dimethyl sulfoxide, etc. are the electron acceptors (Figure 1.11).

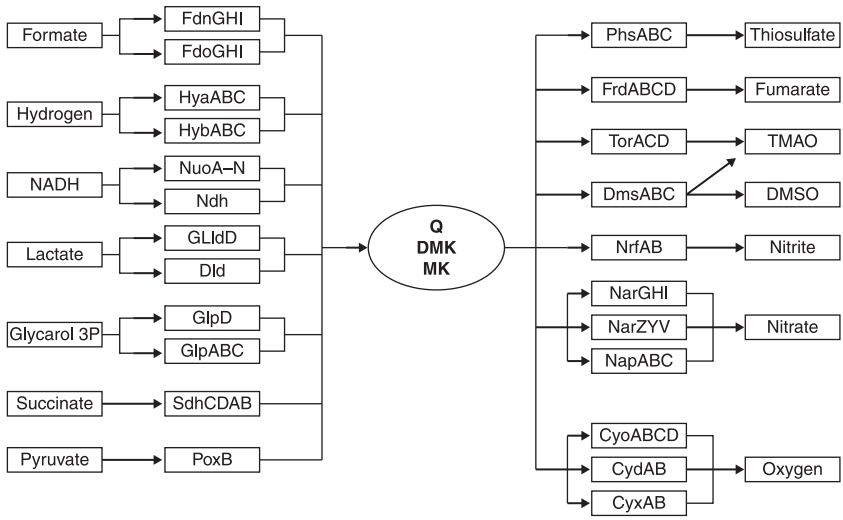


Figure 1.11 Modularity of respiratory chains (Gennis and Stewart, 1996)

This process is coupled to the formation of a **proton motive force** (**PMF**), which is utilized for ATP generation from ADP. *E. coli* can make two different NADH dehydrogenases and two terminal oxidases, as shown in Figure 1.11. Figure 1.12 shows the electron flux by such pathways, where NDH-I is a primary proton pump and results in translocating $2H^+/e^-$, whereas NDH-II is not coupled ($H^+/e^- = 0$). The two cytochrome oxidases are also different in their efficiency of proton translocation, with $H^+/e^- = 2$ for cytochrome bo_3 (Cyo), and $H^+/e^- = 1$ for cytochrome *bd* (Cyd). This indicates that the number of protons delivered to the periplasm varies from 1 to 4 per electron, depending on which pathways are utilized in relation to oxygen levels. It may be considered that the maximum value

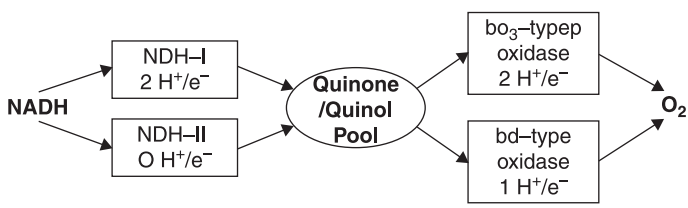


Figure 1.12 Proton translocating values per electron in the respiratory chain

Published by Woodhead Publishing Limited, 2013

is obtained for the case of NDH-I and Cyo under fully aerobic conditions, and the minimum value is obtained for NDH-II and Cyd under micro-aerobic conditions, but these regulations are more complex, as will be explained in Chapter 3. Note that the affinity to oxygen is high for Cyd (K_m (O_2) = 0.3 μM) (Mason et al., 2009), while it is low for Cyo ($K_m(O_2)$ = 6.0 μM) (Mason et al., 2009), but the reaction rate such as V_{max} is the reverse (V_m = 218 mol O/mol cytochrome bd/s for Cyd (Bekker et al., 2009), and V_m = 225 mol O/mol cytochrome bd/s Cyo) (Sato-Watnabe et al., 1998). Note also that the important function of the respiratory chain is the maintenance of the redox balance and the regeneration of NAD^+ from NADH, in addition to its bioenergetic efficiency (Gennis and Stewart, 1996).

One of the major functions of the respiratory chain is to generate a proton electrochemical gradient, referred to as the proton motive force (PMF), across the cytoplasmic membrane. The respiratory system is designed to oxidize a wide variety of substrates and to utilize different terminal electron acceptors (Figure 1.11) (Gennis and Stewart, 1996). As shown in Figure 1.12, NDH-II has no transmembrane elements, and all the chemistry occurs on the cytoplasmic side of the membrane, and the bioenergetic contribution of this enzyme to the PMF is negligible. SDH in the TCA cycle contains membrane-spanning subunits, but the protons used in the reduction of ubiquinone come from the cytoplasm and do not generate PMF. The Frd is also unlikely to contribute to the PMF. Figure 1.13 shows the electron transfer and the proton translocation across the membrane (Gennis and Stewart, 1996).

Quinones are widely distributed in nature. These are lipid-soluble components of membrane-bound electron-transport chains, where quinone is present not only in the inner mitochondrial membrane but also in various organs, which implies its role in other than respiratory electron transport (Soballe and Poole, 1999). Quinones may be subdivided into two groups, one of which comprises a benzoquinone called ubiquinone or coenzyme Q, with the latter (CoQ) being mostly used in biochemical and clinical research. Ubiquinone is expressed either as UQ or UQ_n, where n refers to the number of isoprenoid units in the side chain (Figure 1.14). The second group contains the naphthoquinones menaquinone (vitamin K_2, MK, or MK_n) and demethyl/menaquinone (DMK or DMK_n).

Animal cells synthesize UQ only, and MK is obtained from the diet (Soballe and Poole, 1999). In prokaryotes, MK is utilized in pyrimidine biosynthesis under anaerobic conditions (Gibson and Cox, 1993).

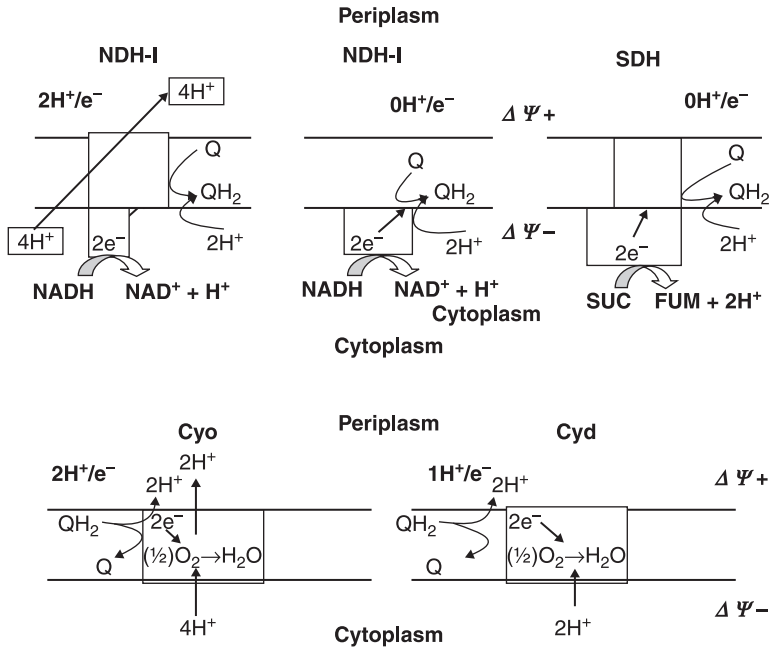


Figure 1.13 Schematic illustration for the electron transfer and proton translocation

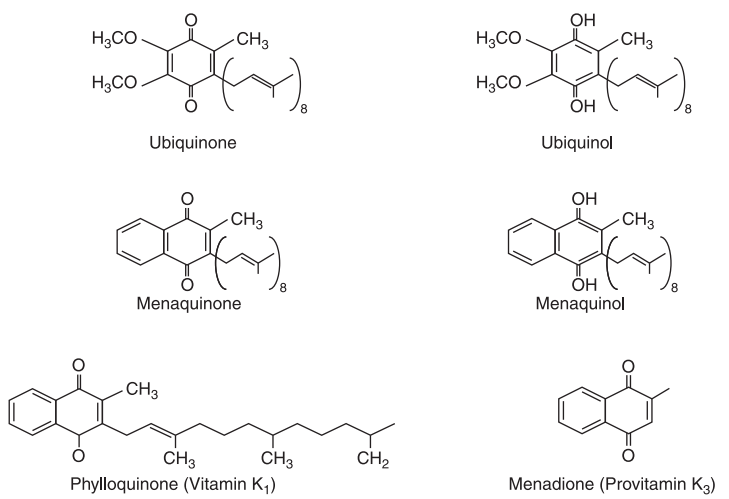


Figure 1.14 Chemical structure of quinones (Mobious et al., 2010)

Published by Woodhead Publishing Limited, 2013

Humans and some plants, such as tobacco plants, contain CoQ_{10}, while prokaryotes, such as *E. coli* and eukaryotes such as yeast, have UQ_8, MK_8, and DMK_8 as well as other side chains. Most Gram-positive bacteria and anaerobic Gram-negative bacteria contain MK only, whereas most of the aerobic Gram-negative bacteria contain exclusively UQ (Soballe and Poole, 1999). Both types of UQ and MK are found in facultative anaerobic Gram-negative bacteria. Pyloquinone (vitamin K_1), a naphthoquinone with a largely saturated side chain (Figure 1.14), is formed mainly in green plants and plays an important function in blood coagulation (Soballe and Poole, 1999). Plastoquinone (PQ_n) is a benzoquinone that acts as a primary electron carrier in the photosynthetic tissues of higher plant as well as in cyanobacteria.

Plumbagin and juglone are naphthoquinones that are excreted by plants to poison predators. The lack of an isoprenoide side chain increases their quinone solubility and they impose severe oxidative stresses on the cell by intercepting electrons from membrane-bound electron carriers and transferring them to molecular oxygen to reduce superoxide (Soballe and Poole, 1999). Plumbagin and juglone are used to induce oxidative stress or as respiratory inhibitors. Menadione is often used as an electron donor in studies with quinone-dependent oxidoreductase, a redox mediator, or as a superoxide-generating agent (Soballe and Poole, 1999).

UQ was discovered independently, where its role is that of a respiratory hydrogen (or proton plus electron) carrier between NADH dehydrogenase or succinate dehydrogenase and cytochrome systems. Reduction and oxidation of UQ involve 2-electron transfers at the quinone nucleus associated with the addition or release of two single H^+ to form ubiquinol (UQH_8) and UQ, respectively. These reactions are important for both linear electron transfer and transmembrane H^+ translocation (Soballe and Poole, 1999).

Removal or transfer of a single electron and H^+ gives the ubisemiquinone radical (UQ^{*-}). This radical may be stabilized when bound to a protein in association with UQH_2 oxidase, and cytochrome bo' in *E. coli* (Ingledeu et al., 1995; Soballe and Poole, 1999).

An important property of UQ is its hydrophobicity, which allows free movement in the membrane. The biosynthesis of UQ is shown in Figure 1.15, where the nucleus is derived from chorismate, whereas the prenyl side chain is derived from prenyldiphosphate, and the methyl groups are derived from S-adenosylmethionine.

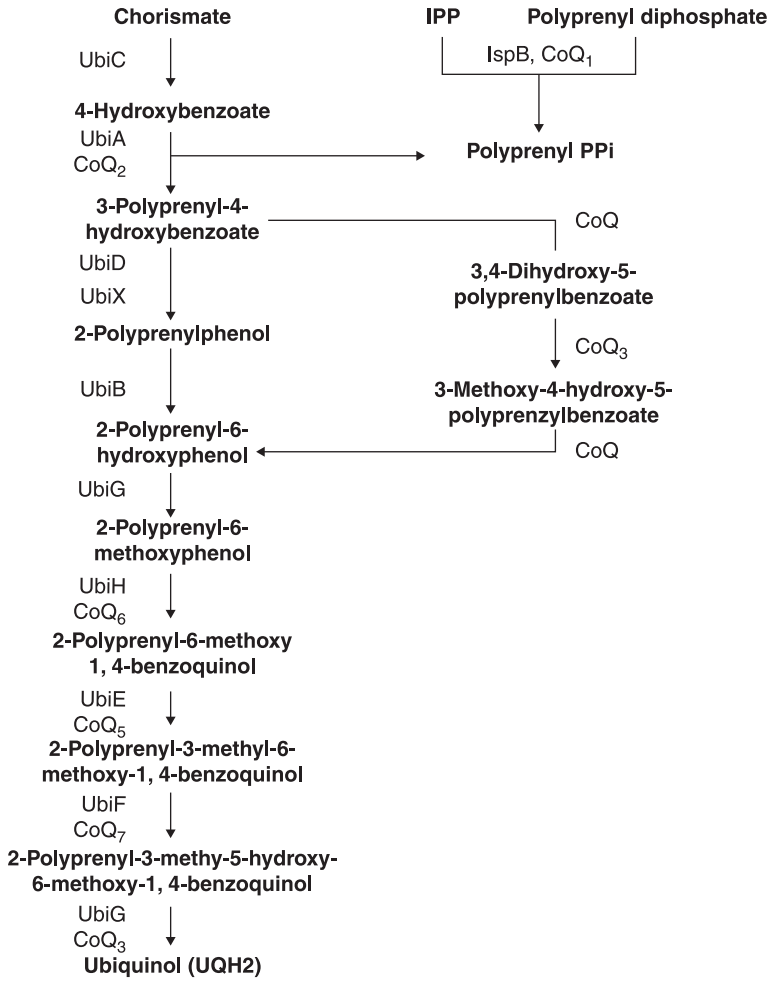


Figure 1.15 Pathway of UQ biosynthesis in *E. coli* (Soballe and Poole, 1999)

1.5 Anaerobic metabolism

In the absence of oxygen or other electron accepters, the respiratory chain cannot be utilized, and thus ATP is generated via substrate level phosphorylation through the process of degradation of the carbon source in the metabolic pathways. Under such fermentation conditions, cells such as *E. coli* excrete metabolites such as lactate, ethanol, succinate, and formate (also CO_2 and H_2) as well as acetate, where the relative production

Published by Woodhead Publishing Limited, 2013

rates for these metabolites are governed by the demand for redox neutrality (Figure 1.16). The succinate is formed from PEP via Ppc. PYR serves as a common substrate for pyruvate formate-lyase (Pfl) and the pyruvate dehydrogenase complex (PDHc), and this branch point involves the cleavage of PYR. The activity of *pfl*, which encodes Pfl, is under the control of such global regulators as ArcA and Fnr in *E. coli*, and becomes active at lower oxygen concentrations, whereas *aceE,F*, which encode α and β subunits of PDHc, are repressed by ArcA under oxygen limited conditions. At the branch point of AcCoA, the product of both Pfl and

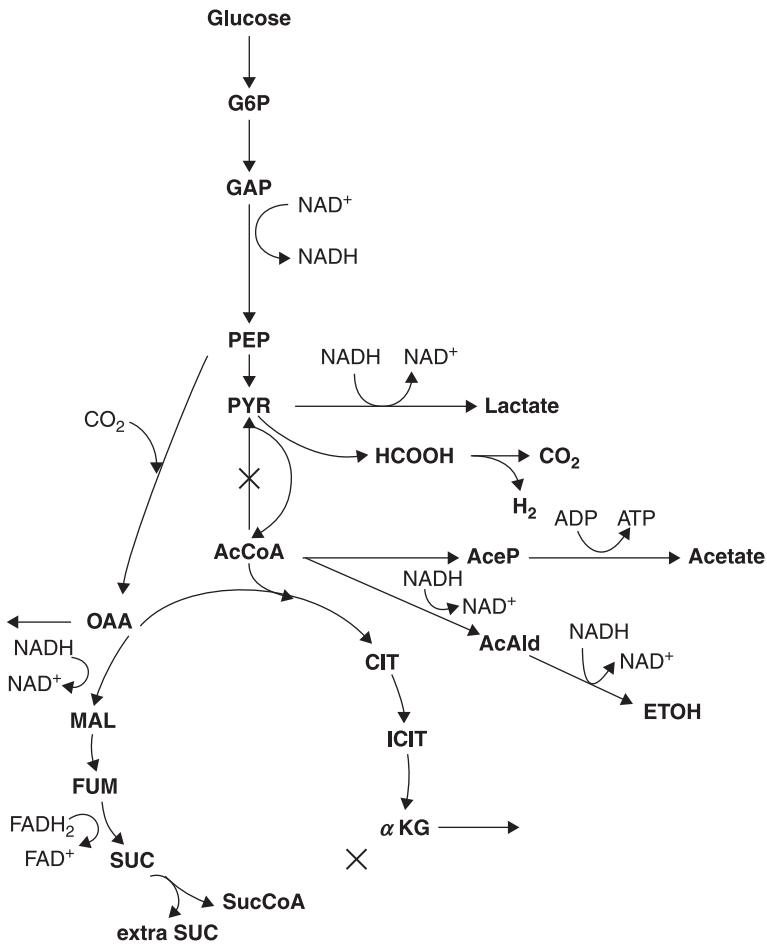


Figure 1.16 Anaerobic pathways

PDHc reactions, AcCoA is converted to either acetate or ethanol, or subsequently undergoes further oxidation in the TCA cycle up to α KG (Figure 1.16).

Anaerobic metabolism is closely related to fermentation, but the meaning of 'fermentation' has been changed during the past hundred years. Pasteur may have used the terms 'cell' and 'ferment' interchangeably when referring to the microbe back in 1857 (Doelle, 1975). The term 'fermentation' originated from wine-making, thus becoming associated with the idea of cells, gas (CO_2) production, and the production of organic by-products (Doelle, 1975). Under anaerobic conditions, energy generation is limited only by substrate level phosphorylation and/or anaerobic respiration, and thus the specific glucose consumption rate is increased to enhance energy production through the EMP pathway (Koebman et al., 2002). The possible fermentation pathways are given in Figure 1.16, where NADH re-oxidation is critical for regeneration of NAD^+ for the metabolism to continue. From research on electron transport systems of microbial metabolism, it may be reasonable to use the term 'fermentation' for the processes that have organic compounds as terminal acceptors (Doelle, 1975). However, with this definition, acetic acid bacteria may not be fermentative but respire aerobically. It may be better to extend the definition of fermentation to also include acetic acid fermentation.

1.6 Anaerobic respiration

The reduction of nitrate to nitrite and then ammonia appears widely in bacteria, archaea, and as plants. *E. coli* and enteric bacteria can combine such reduction processes with electron transport systems (Figure 1.17). Note that nitrate assimilation for anabolism occurs in the cytoplasm, but it can occur in the cytoplasm or periplasm or both, depending on the growth conditions. Moreover, unlike nitrate assimilation, it is strictly an anaerobic process in enteric bacteria, where the expressions of nitrate and nitrite reductase genes are tightly repressed in the presence of oxygen. These are induced under anaerobic conditions and further regulated by the availability of nitrate and nitrite. The regulatory reduction of nitrate to ammonia occurs in many electron-rich environments, such as anoxic marine sediments and sulfide-rich thermal vents, the human gastrointestinal tract, and the bodies of warm-blooded animals.

Published by Woodhead Publishing Limited, 2013

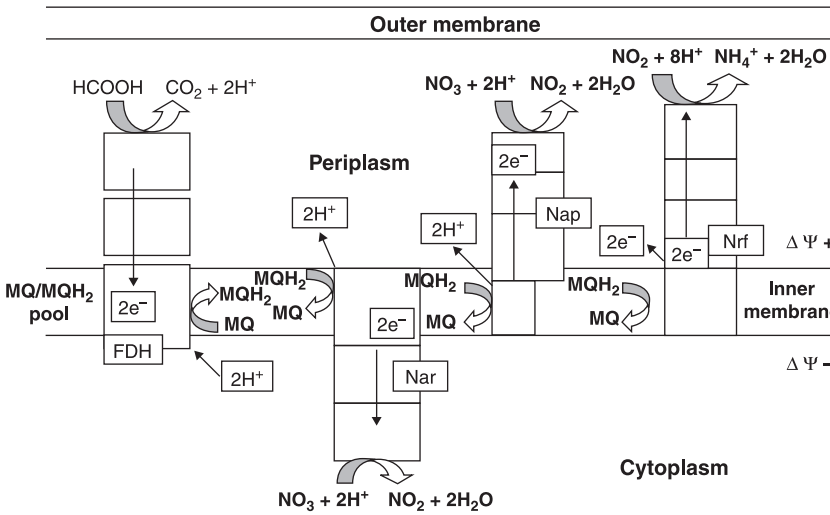


Figure 1.17 Nitrate respiration

1.7 Photosynthesis

In typical plant cells, carbohydrate is formed from CO_2 and water in the atmosphere, with the aid of light energy from the sun. This process is called photosynthesis, where light energy is stored as ATP and NADPH, which are utilized to convert CO_2 to 3-phosphogrycerate (3PG), where 3PG is in turn converted to hexose phosphate. The photosynthetic organisms are not restricted to plant cells but also include photosynthetic bacteria such as cyanobacteria and algae, etc.

Most autotrophic organisms fix CO_2 by the reaction catalyzed by riburose 2-phosphate carboxylase, where CO_2 and H_2O are converted to 3PG. This is an important step in the Calvin (or Calvin-Benson) cycle.

The overall photosynthesis reactions may be expressed as:

$$6CO_2 + 6H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + 6O_2 \quad (1.40a)$$

or

$$CO_2 + H_2O \xrightarrow{\text{light}} (CH_2O) + O_2 \quad (1.40b)$$

where (CH_2O) denotes carbohydrate.

The first step of photosynthesis is called the light reaction or light phase, where light energy is stored in the form of ATP and NADPH. In this stage, a hydrogen atom is removed from the water molecule and then utilized to reduce $NADP^+$, and the oxygen molecule is left as it is. At the same time, ADP is phosphorylated to form ATP. The reaction during this light phase may be expressed as:

$$H_2O + NADP^+ + P_i + ADP \xrightarrow{\text{light}} 0.5O_2 + NADPH + H^+ + ATP \quad (1.41)$$

In the second stage of photosynthesis, glucose or carbohydrate are formed from CO_2 by utilizing NADPH and ATP produced during the first stage. This stage is known as the dark reaction phase or dark phase, where NADPH is re-oxidized to $NADP^+$, and ATP is hydrolyzed to ADP and P_i as:

$$CO_2 + NADPH + H^+ + ATP \rightarrow (1/6)Glc(CH_2O) + NADP^+ + ADP + P_i \quad (1.42)$$

Photosynthesis occurs in multiple membranes in prokaryotic organisms such as cyanobacteria, while it occurs in organelles as chloroplasts in eukaryotic organisms. The chloroplast forms the complex net structure called the thylakoid membrane, where NADPH and ATP are formed. Variously colored elements are embedded inside the thylakoid membrane to capture photo energy, where green chlorophyll is the most common color element, and light absorption by chlorophyll molecules excites electrons. The excited chlorophyll molecule generates a photon by the fluorescence process, and returns it to its original state. The excited chlorophyll gives the electron a series of enzymes, and ATP is generated through transfer of the electron via this chain of enzymes. This ATP generating process is called photo-phosphorylation. The electron carriers in this process are ferodoxin and some cytochrome. The light phase of photosynthesis consists of two photo-systems. Photo-system I (PS I) is excited by light with a lower wavelength than 700 nm, and produces NADPH. Photo-system II (PS II) requires light with a wavelength lower than 680 nm, and decomposes H_2O into $(1/2)O_2$ and $2H^+$. Here ATP is generated in accordance with the electron flow from PS I to PS II.

In the second stage of photosynthesis, CO_2 is converted to carbohydrate using ATP and NADPH obtained during the light reaction phase. This reaction can be considered as three reactions, namely: i) CO_2 fixation; ii) conversion of fixed CO_2 to carbohydrate; and iii) regeneration of the CO_2 acceptor molecule. In the first process of CO_2 fixation, the

Published by Woodhead Publishing Limited, 2013

reduced PP pathway, the C_3 cycle pathway, is so-called because the first intermediate is a three-carbon molecule, typically called the Calvin (or Calvin-Benson) cycle.

In plant cells, CO_2 is fixed by photosynthesis through a structure on the leaf called the stromata. In the first step reaction of the Calvin cycle, 2 moles of 3 PG are formed from 1 mole of CO_2 and 1 mole of riburose 1,5-bisphosphate (Ribu1,5 BP), where this reaction is irreversible and catalyzed by riburose 1,5-bisphosphocarboxylase oxigenase or by the more popular named RubisCo. The Calvin cycle is shown in Figure 1.18, where 3PG is metabolized in the reverse direction to the glycolysis, similar to gluconeogenesis, and 1,3 BPG is formed from 3 PG using ATP by the reaction catalyzed by Pgk, and GAP is formed from 1,3 BPG by the re-oxidation of NADPH (instead of NADH) catalyzed by the isozyme of GAPDH. Then, part of GAP is converted to sucrose or starch, and the rest is used for the regeneration of Rib1,5 BP. In summary, the overall Calvin cycle reaction can be expressed as:

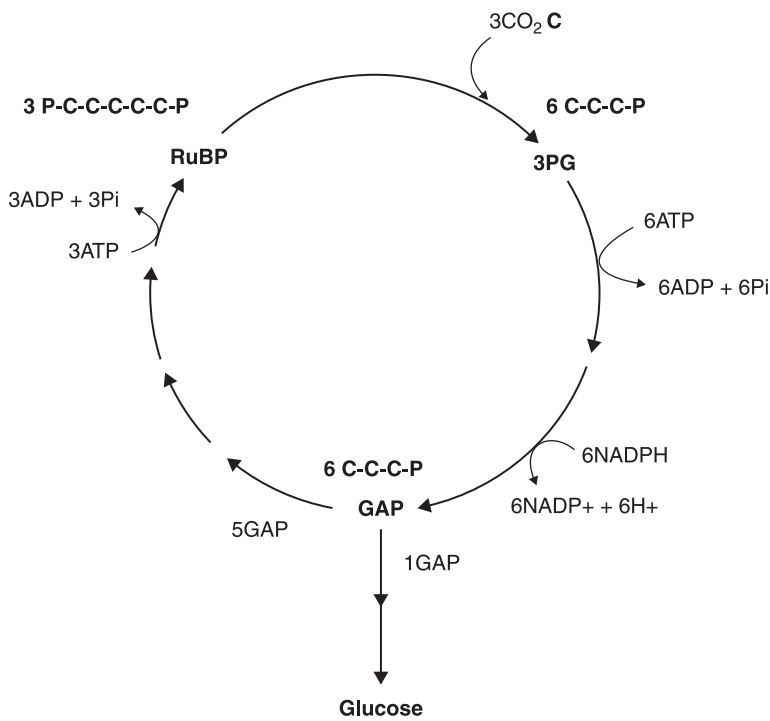


Figure 1.18 Calvin-Benson cycle

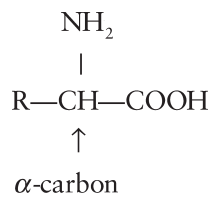
$$3CO_2 + 9ATP + 6NADPH + 5H_2O \rightarrow 9ADP + 8P_i + 6NADP^+ + GAP \text{ or } DHAP \quad (1.43)$$

Note that RubisCo catalyzes the carboxylation and oxygen addition, as the alternative name implies. In the reaction of O_2 addition, 1 mole of 3 PG and 1 mole of phosphogrycerate are formed. The 3 PG formed at the oxygen addition reaction of Rib1,5BP enters the Calvin cycle, and 2 moles of 2 PG(C_2) are oxidized to produce 1 mole of CO_2 and 1 mole of 3 PG (C_3), and these again enter the Calvin cycle. During the oxygen addition process, NADH and ATP are used. After light-dependent O_2 uptake is catalyzed by RubisCo, this is released as CO_2 by the phospho-glycerate metabolism. This is called **photorespiration**.

1.8 Amino acids synthesis

Amino acid synthesis is important for the cell synthesis. Figure 1.19 shows 20 amino acids and their precursors.

Note that the chemical structure of amino acids is:



As can be seen, amino basis ($-NH_2$ or $-NH_3^+$) connects to the α carbon (next to the carboxyl base). An amino acid can produce H^+ from COOH, and thus is an acid. Different amino acids come from different structures of R. Let us now consider how these amino acids are synthesized from their precursors.

1.8.1 Alanine

Pyruvate is the precursor for alanine, where pyruvate is reductively converted to form L-alanine by alanine dehydrogenase, or by the transfer of the amino base to pyruvate by transaminase. L-alanine is converted to D-alanine by a racemase reaction, and is utilized as the constitutent for the cell wall, etc. (Figure 1.20).

Published by Woodhead Publishing Limited, 2013

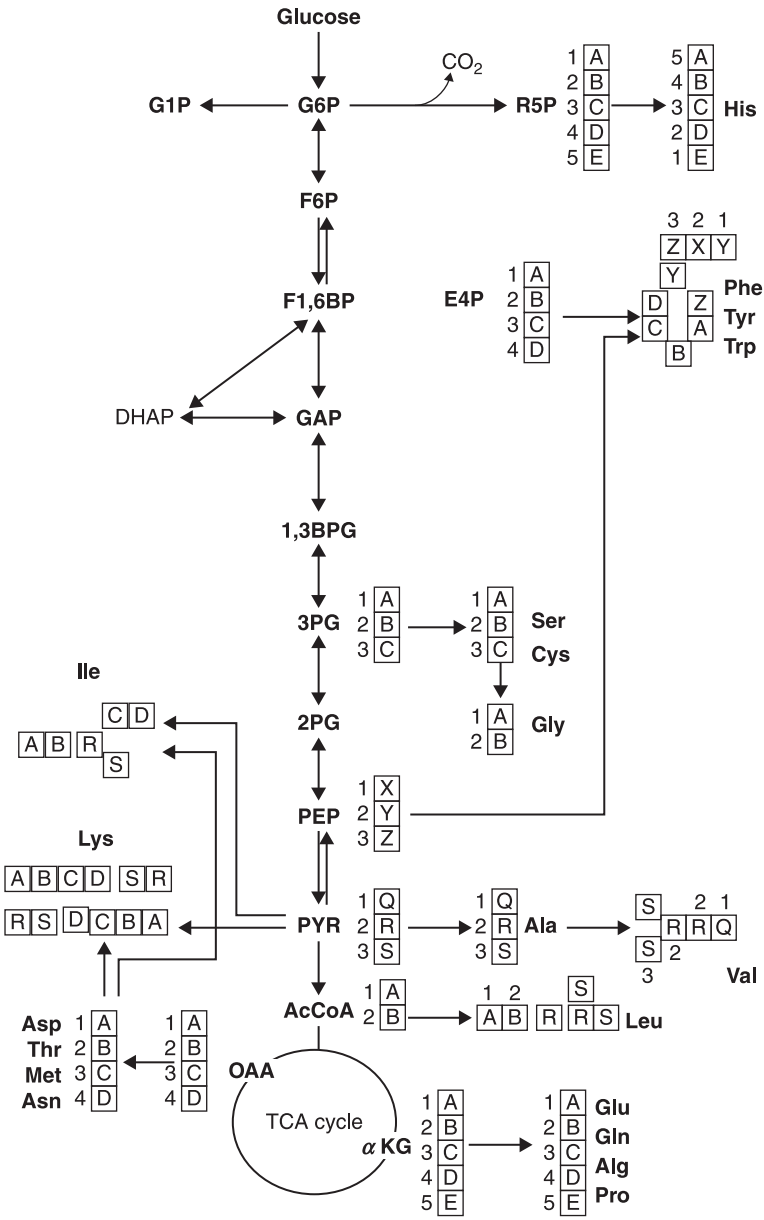


Figure 1.19 Amino acid synthesis from their precursors

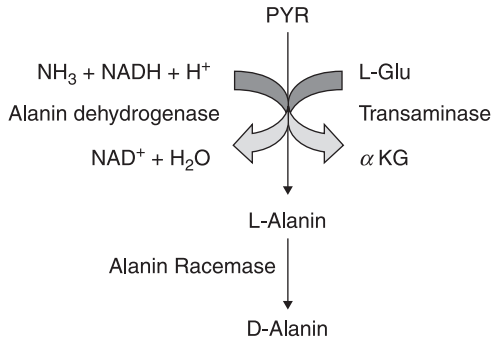


Figure 1.20 Alanine synthesis from PYR

1.8.2 Valine, leusine, isoleusine

Valine, leusine, and isoleusine are the branch chain amino acids, where the isoleusine synthesis pathway from 2-oxobutyrate and the valine synthesis pathway from pyruvate are catalyzed by the common enzyme (Figure 1.21). The final step reactions for valine, leusine, and isoleusine are amine transfer reactions, which are all catalyzed by the branch chain amino-transferase. Moreover, each amino acid synthetic pathway is under feedback control by the final product of amino acids, where isoleusine inhibits threoninehydratase, valine inhibits acetohydroxylic acid synthase, and leusine inhibits isopropyl malic acid synthase. Note that threonine hydratase, acetohydroxylic acid synthase, and branched chain amino acid transaminase are regulated by the multivarent control.

1.8.3 Glutamate, glutamine

Glutamic acid (Glu) is synthesized from α KG in the TCA cycle by glutamate dehydrogenase (GDH) with NH_3 and NADPH (Figure 1.22). The glutamic acid produced inhibits GDH and also controls enzyme synthesis. Note that glutamic acid regulates the synthesis of Ppc and CS. Gutamine is synthesized from glutamate by glutamine synthetase (GS) with NH_3 and ATP (Figure 1.22). Glutamic acid may be also formed from glutamine by glutamate synthase (GOGAT) with NADPH when the NH_3 concentration is low and limiting. The ammonia assimilation or nitrogen regulation pathways are important, which will be explained in more detail in Chapter 3.

Published by Woodhead Publishing Limited, 2013

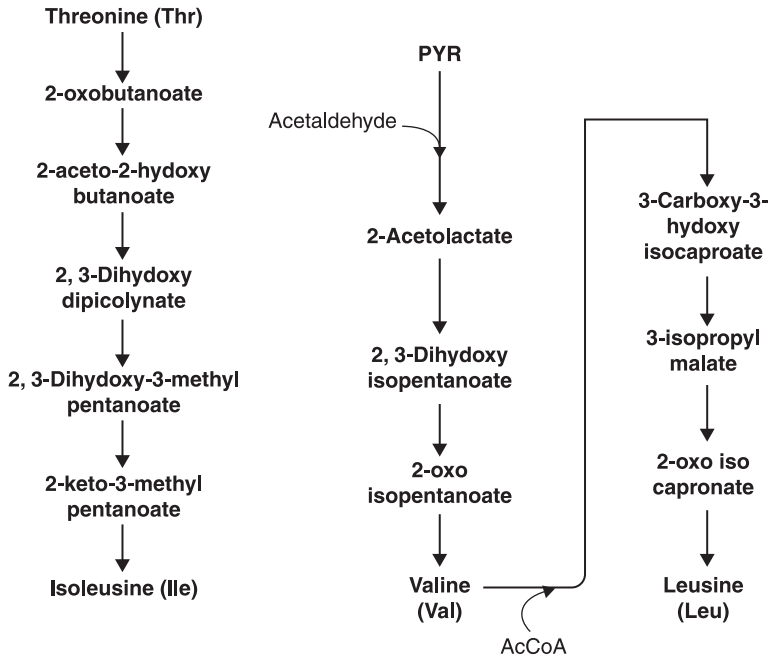


Figure 1.21 Valine, leusine, and isoleusine biosynthesis

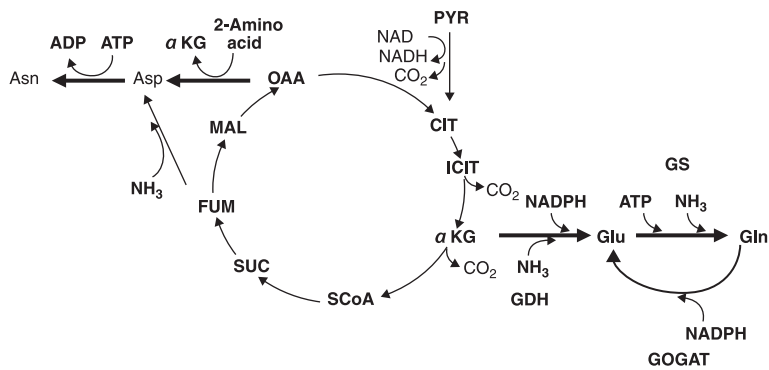


Figure 1.22 Glutamate and glutamine synthesis as well as aspartate and asparagine synthesis

1.8.4 Proline

Proline is synthesized from glutamate, where glutamate is converted to glutamate 5-semialdehyde by glutamate kinase and 5-glutamil phosphate

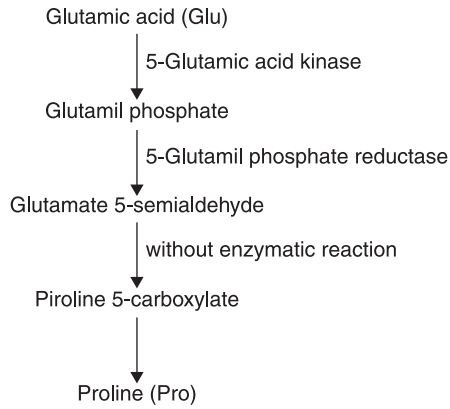


Figure 1.23 **Proline biosynthesis from glutamate**

reductase, and this becomes proline 5-carboxylic acid and then proline. The first reaction step of these reactions is inhibited by proline (Figure 1.23).

1.8.5 Arginine, ornitine, and citorline

As shown in Figure 1.24, ornitine is formed by five reaction steps, citorline by six steps and arginine by eight steps of reactions from glutamic acid. These sets of reactions are also inhibited by arginine.

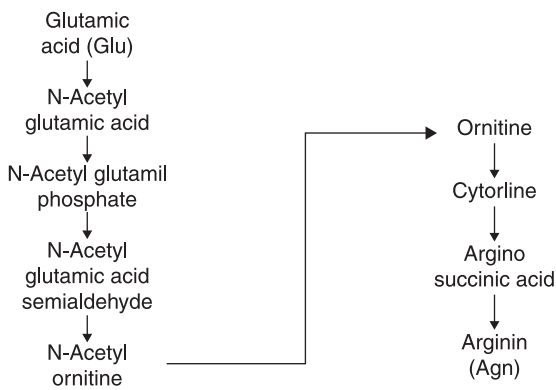


Figure 1.24 **Arginine, ornitine, and citorline synthesis pathway**

Published by Woodhead Publishing Limited, 2013

1.8.6 Aspartate and asparagine

Aspartic acid (Asp) is synthesized from OAA in the TCA cycle by aspartate transaminase. As stated before, OAA is synthesized from PEP or PYR by the anaplerotic reaction of Ppc (or Pyc) with fixation of CO_2. In bacteria such as *E. coli*, Ppc is inhibited by Asp. Asparagine (Asn) is synthesized by asparagine synthase from Asp (Figure 1.22).

1.8.7 Lysine, threonine, and methionine

Figure 1.25 shows the lysine (Lys) synthetic pathways, where the bacterial synthesis of lysine takes place via the diaminopimelate (DAP) pathway. L-aspartate (Asp) is formed from OAA by transamination, as stated above. Asp is then activated via phosphorylation by aspartokinase and reduced to form L-aspartate semialdehyde in the first two steps. L-aspartate semialdehyde is at a branch point to enter either threonine, methionine, and isoleusine syntheses or lysine synthesis. Dihydrodipicolinate (DHPS) and dihydrodipicolinate reductase (DHPR) catalyze the third and fourth

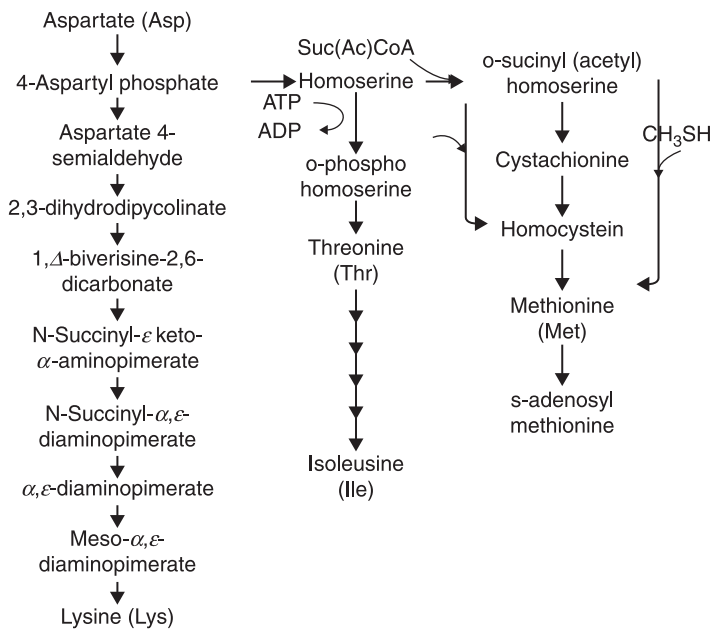


Figure 1.25 Lysine, threonine, and methionine biosynthesis

steps in the lysine synthetic pathway, respectively, and are the enzymes that commit flux to the biosynthesis of meso-diaminopimelate (DAP) and lysine. The synthesis of DAP from L-tetrahydrodipicolinate (THDP) is accomplished by three separate routes: the succinylase and acetylase pathways, in which *N*-succinylated or *N*-acetylated intermediates are generated, and the infrequently encountered dehydrogenase pathway. The synthesized meso-DAP can be either used for cell wall synthesis or decarboxylated to L-lysine catalyzed by DAPDC (DAP decarboxylase).

In threonine synthesis, homoserine is first formed from 4-aspartyl phosphate (ASA) by homoserine dehydrogenase. The homoserine is then converted to threonine (Thr) by homoserine kinase and threonine synthase. The limiting pathways for threonine synthesis are the reactions catalyzed by aspart kinase (Ask) and homoserine dehydrogenase, where isozyme I of Ask is threonine sensitive for *E. coli* K12. Moreover, a set of genes form an operon, and a series of enzymes are under multi-varent repression by threonine and isoleusine. In the case of *Corynebacteria*, ASA is under concerted repression when threonine and lysine co-exist, and homoserine dehydrogenase is subject to strong inhibition only by threonine.

Methionine is first formed from homoserine by homoserine-o-succinyl (acetyl) transferase. Then it is converted to cystachionine by cystachionine-γ-synthase by introducing a sulfur molecule. Finally, methionine is synthesized by introducing methyl basis via homocystein. In general, homoserine-o-succinyl (acetyl) transferase is repressed by methionine.

1.8.8 Aromatic amino acids

Aromatic amino acids, such as tryptophane (Trp), phenylalanine (Phe), and tyrosine (Tyr), are formed from E4P in the PP pathway and PEP in the glycolysis. The first reaction of this synthesis is catalyzed by deoxyalabino hepturose phosphate synthase (DAHPS), where this is an important regulatory enzyme. Then shikimic acid (Shik) is formed after four steps from the first reaction, and chorismic acid (CM) is formed after seven steps of reactions from the first reaction (Figure 1.26). From chorismate, antranil acid is formed by antranil acid synthase, and Trp is formed after several reaction steps from this. However, prephenic acid (PA) is formed from CM by corismic acid mutase, and then Trp is formed from PA by prephenic acid dehydrogenase and tyrosine transaminase. Phenylalanine is formed from PA by prephenic acid dehydrogenase and tyrosine transaminase.

It is known that different regulation mechanisms exist, depending on the microorganisms. Figure 1.27 shows three typical regulation patterns

Published by Woodhead Publishing Limited, 2013

Figure 1.26 **Aromatic amino acid synthesis pathways**

(Nagai et al., 1996). Figure 1.27a shows the sequential control pattern typically seen in *B. subtilis*, where Phe and Tyr are formed by the branched pathway from PA, and each first enzyme is inhibited by the corresponding products, and the accumulation of CM inhibits DAHPS and shikimate kinase. This control scheme acts sequentially from the end products. Figure 1.27b shows the regulation scheme typically seen in *E. coli.*, etc., where the first enzyme DAHPS from E4P and PEP consist of the three enzymes of I, II, III, each sensitive to Phe, Tyr, and Trp, respectively. Figure 1.27c shows the regulation scheme typically seen in *B. flavum* or *Corynebacteria*, where this scheme is known as the priority synthesis. Namely, antranyl acid synthesis is inhibited by the accumulation of Trp, prephenic acid dehydratase is inhibited by the accumulation of Phe, and DAHPS is inhibited only when both Phe and Tyr have accumulated.

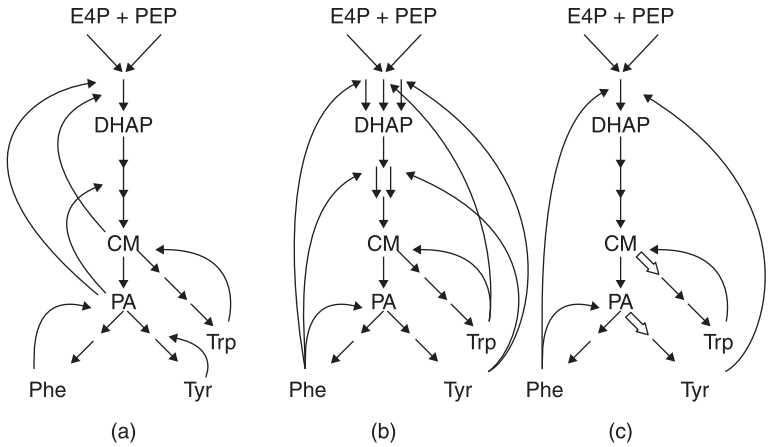


Figure 1.27 **Several control schemes for aromatic amino acid biosynthesis**

1.8.9 Serine, glysine, and cystein

As shown in Figure 1.28, serine is formed from 3PG in the EMP pathway via three step reactions. The first enzyme phosphoglycerate dehydrogenase

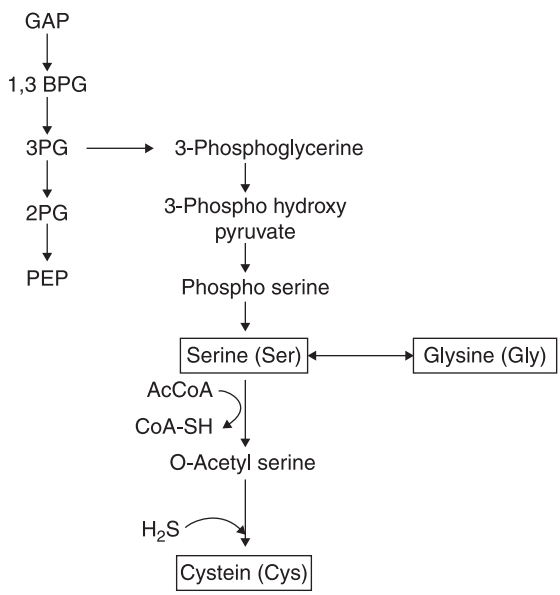


Figure 1.28 **Serine, glycine, and cystein synthesis pathways**

Published by Woodhead Publishing Limited, 2013

of these reactions is inhibited by serine. Cystein is formed from serine by the incorporation of H_2S to acetylphosphate formed by acetyltransferase. The mutual exchange takes place between serine and glysine by serine hydroxymethyl transferase. However, glysine can be formed from threonine by transaldorase. Moreover, glycine can be formed from inorganic ammonia salt and 5,10 methylene tetrahydro folate by glycine synthase. Note that cystein contains sulfur.

1.8.10 Histisine

As shown in Figure 1.29, histisine is formed from R5P in the PP pathway, where phospho ribosilpyrophosphate (PRPP) is formed from R5p by the reaction catalyzed by ribose phosphate pyrophosphokinase, and phosphoribosil ATP is formed by ATP-phosphoribosil transferase, where the first step enzyme ATP-phosphoribosil transferase is under feedback inhibition by histisine.

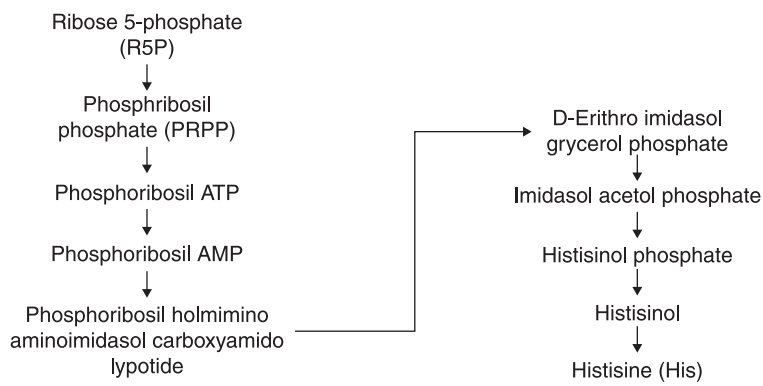


Figure 1.29 Histisine synthesis pathways

1.9 Nucleic acid synthesis and its control

1.9.1 Prine type nucleic acid synthesis

ATP or GTP are synthesized from R5P in the PP pathway, as shown in Figure 1.30, where inosine 5′-monophosphate (IPM) is formed from 5-phosphoribosil pylophosphate (PRPP) by 10 step reactions. It has been

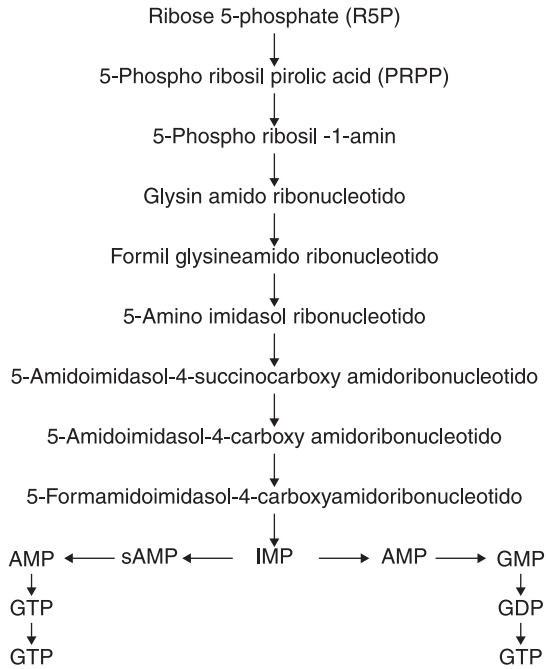


Figure 1.30 **Nucleic acids synthesis pathways**

known that four bases, such as hypoxantine, adenine, guanine, and xantine and their nucleoside or nucleotide, are interchanged.

1.9.2 Pyrimidine type nucleic acid synthesis

The first enzyme for the synthesis of pyrimidine type of nucleic acid is carbamyl phosphate synthase. The uridine-5′-monophosphate (Urydylate: UMP) is synthesized by six steps. UMP or cytidine acid (cytidine 5′-nucleotidase: CMP) is dephosphorylated by 5′-nucleotidase or phosphatase to become each nucleoside, and decomposed to the corresponding bases by pyrimidine nucleoside phosphorylase. Inversely, bases become nucleosides by the reverse reaction of phospholylase, while nucleotides are synthesized by the salvage synthesis by each kinase. Moreover, cytosine derivatives such as CTP are formed from UTP, where these are converted to urasil derivatives by each corresponding aminase.

Published by Woodhead Publishing Limited, 2013

1.10 Fatty acid metabolism and degradation

Fatty acid is first activated by ATP and converted to AcylCoA by the action of AcylCoA synthase. Long-chain AcylCoA is decomposed by releasing AcCoA one by one. This degradation pathway is called the β-oxidation pathway, since the β carbon (C-3) of fatty acid is oxidized. Fatty acid is the substance with a highly reductive state. As shown in Figure 1.31, $FADH_2$ or NADH is generated by β-oxidation, and these can be utilized for ATP synthesis by oxidative phosphorylation. Thus, a fatty acid with an even number of carbons is all degraded into AcCoA and goes into the TCA cycle, while that with an odd number of carbons generates propionylCoA, which is carboxylazed and converted to SucCoA via methyl malonylCoA and goes into the TCA cycle.

The synthesis and the degradation by β-oxidation occur along totally different pathways. In mammalian cells, β-oxidation of fatty acid is made

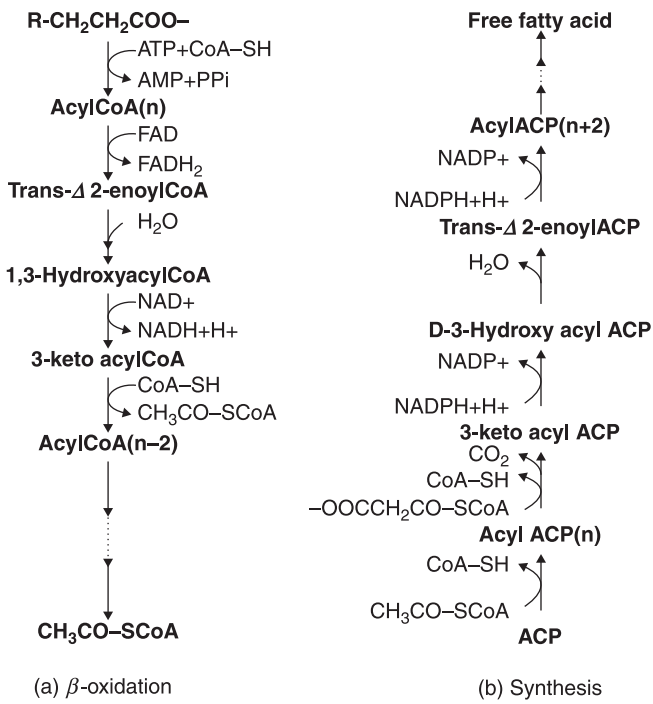


Figure 1.31 **β oxidation and biosynthesis of fatty acid**

in mytochondria, while the synthesis is made in cytosol. The thioester for β-oxidation of fatty acid is a CoA derivative, while in fatty acid synthesis, the intermediate thioester connects to the acyl carrier protein (ACP). The reaction occurs by two carbons in both synthesis and degradation, and two carbon AcCoA is formed in oxidation, while three carbon malonylCoA is required for the synthetic reaction, and two carbons are added to the chain for extension. In this process, CO_2 is released and the reducing equivalent for the reaction is NADPH.

1.11 Phosphotransferase system (PTS)

As can be seen in Figure 1.32, once inside the periplasm through the outer membrane, various sugars can be internalized into the cytoplasm by the PEP: sugar phosphotransferase system (PTS). This system is widespread in bacteria and absent in Archae and eukaryotic organisms (Postma et al., 1996; Saier, 2000). PTS participates in the transport and phosphorylation of a variety of sugars. The system is composed of the soluble and non-sugar-specific protein Enzyme I (EI), encoded by *ptsI* and the phosphohistidine carrier protein (HPr), encoded by *ptsH*. These proteins relay a phosphoryl group from PEP to the sugar specific EIIA and in turn to EIIBC, where EIIC (in some cases also EIID) is an integral membrane protein permease that recognizes and transports the sugar molecules, which are phosphorylated by EIIB. There are 21 different EII complexes encoded in the *E. coli* chromosome, which are involved in the transport of about 20 different carbohydrates (Deutscher et al., 2006).

1.12 Carbohydrate metabolism other than glucose

Glucose metabolism has been explained in Section 1.3 above. Here, other carbon source metabolisms are briefly explained.

1.12.1 Fructose metabolism

There are three pathways for the utilization of fructose (Figure 1.33) (Kornberg, 2001). In the primary pathway, the fructose (Fru) is

Published by Woodhead Publishing Limited, 2013

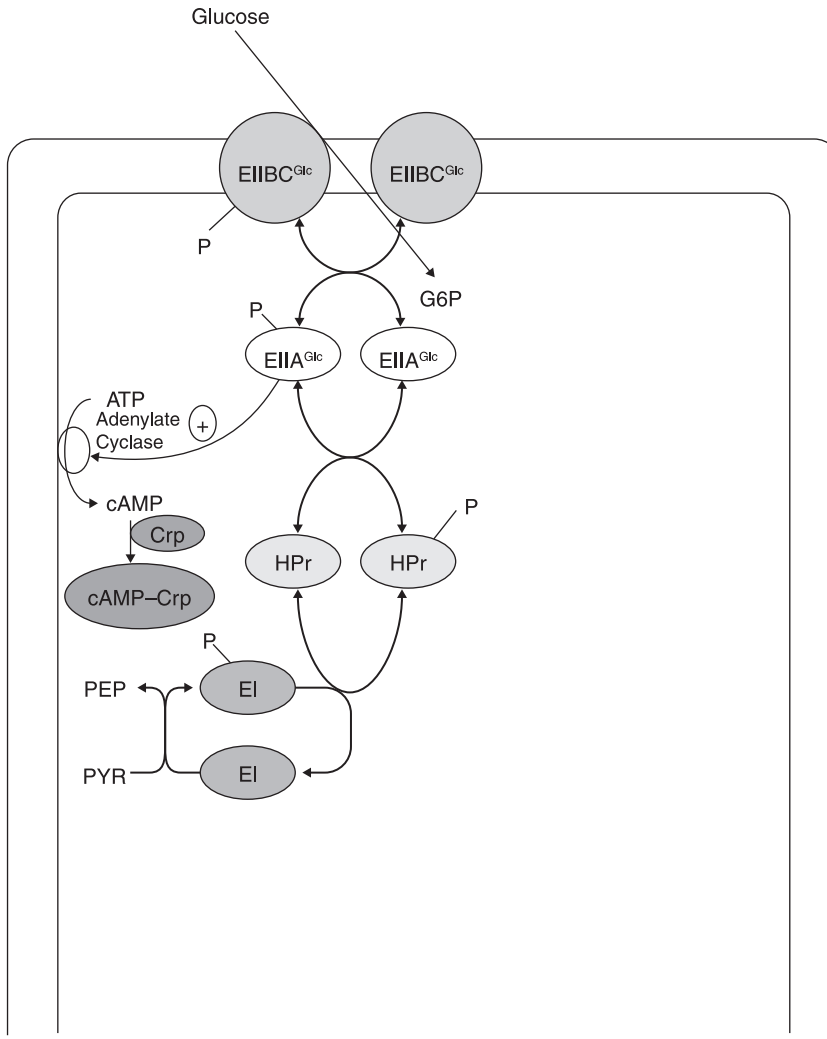


Figure 1.32 Phosphotransferase system (PTS)

transported via the membrane-spanning protein FruA and concomitantly phosphorylated by a PEP: D-fructose 1-phosphotransferase (fructose PTS) system (ATP: D-fructose 1-phosphotransferase, EC 2.7.1.3), which is induced by D-fructose and enters the cell as D-fructose 1-phosphate (F1P), where this process is affected by the transfer of a phosphoryl moiety from PEP to the hexose by the concerted action of two cytoplasmic proteins: EI of PTS and a membrane-associated diphosphoryl protein

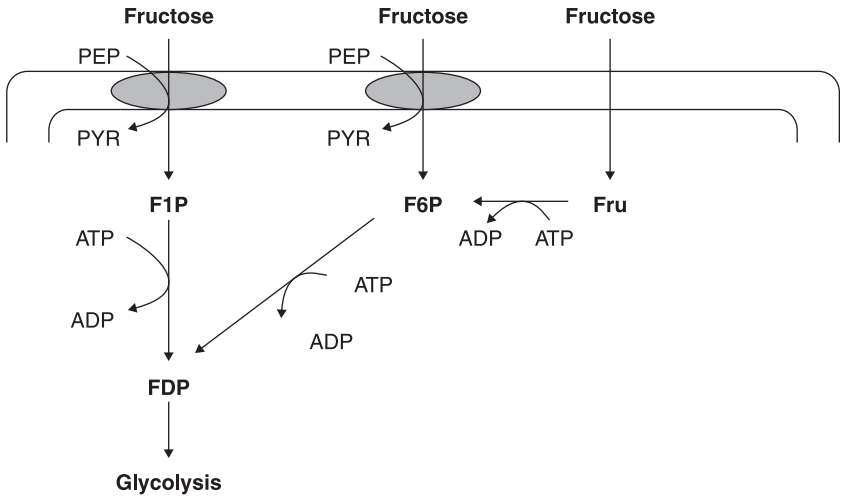


Figure 1.33 Fructose metabolism

(DTP). F1P is then converted to fructose 1,6-diphosphate (FDP) by ATP and by the inducible enzyme D-fructose-1-phosphate kinase (F1PK)(ATP: D-fructose-1-1phosphate 6-phospho-transferase).

In the second pathway, fructose enters the cell via a membrane-spanning protein that has the general ability to recognize sugars possessing the 3,4,5-D-arabino-hexose configuration, which include the permeases for mannose (ManXYZ), glucitol (SrlA), and mannitol (MtlA) (Kornberg, 2001). D-fructose is converted to F6P by a specific sucrose-induced D-fructokinase (ATP: D-fructose 6-phosphotransferase, EC 2.7.1.4), and then converted to FDP by Pfk of the EMP pathway.

In the third pathway, fructose enters the cell by diffusion, using an isoform of the glucose transporter PtsG. Since this mode of entry does not involve the PTS, the free fructose has to be phosphorylated by ATP to become F6P.

1.12.2 Mannose metabolism

The catabolism of mannose can follow two different mechanisms, such as cyclic and non-cyclic mechanisms for the D-isomer, where only the non-cyclic system occurs when L-mannose is the substrate. L-mannose is converted to L-fructose by an isomerase. L-fructose is then phosphorylated at C-1 with ATP by a kinase reaction and L-fructose 1-phosphate is

Published by Woodhead Publishing Limited, 2013

formed, where an aldolase-type cleavage produces DHAP and L-GAP, where this connects to the EMP pathway (Doelle, 1975).

1.12.3 Xylose metabolism

D-xylose is converted to D-xylulose by xylose isomerase (D-xylose keto-isomerase, EC 5.3.1.5) (Figure 1.34). D-xylulose is subsequently phosphorylated by xylulokinase (ATP: D-xylulose 5-phosphotransferase, EC 2.7.1.17) to form D-xylulose 5-phosphate (X5P). Under anaerobic conditions, xylulose reductase (XR) is induced and xylitol and xylitol-5-phosphate are produced, where those may inhibit cell growth (Hausman et al., 1984).

1.12.4 Glycerol metabolism

Glycerol is oxidized to dihydroxyacetone by glycerol dehydrogenase (glycerol: NAD oxidoreductase, EC 1.1.1.6). Dihydroxyacetone is then phosphorylated by a kinase using ATP. Another pathway for glycerol utilization is that glycerol is phosphorylated by glycerol kinase (ATP: glycerol phosphotransferase, EC 2.7.1.30) to form L-glycerol 3-phosphate, which is then converted to GAP.

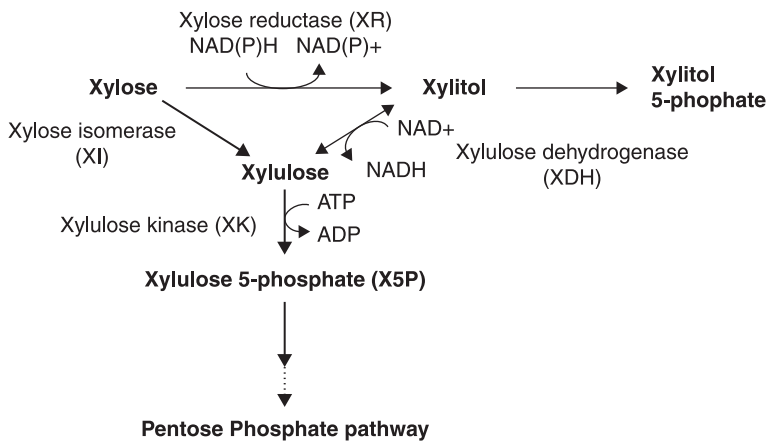


Figure 1.34 Xylose metabolism

1.13 ATP generation under aerobic and anaerobic conditions

Figure 1.35 shows the overall ATP generation under aerobic and anaerobic conditions, which indicates that total ATP generation may be expected to be 38 moles of ATP from 1 mole of glucose by assuming that 1 mole of NADH can be used to generate 3 moles of ATP, while 1 mole of $FADH_2$ can be used to generate 2 moles of ATP generated in the respiratory chain. However, in anaerobic conditions, NADH generated at GAPDH is re-oxidized by producing fermentation metabolites such as lactate, ethanol, and succinate, and only 2 moles of ATP are generated without taking into account ATP generation at Ack for acetate formation (Figure 1.35). Since the specific ATP production rate is correlated with the specific growth rate, the cell growth rate becomes significantly lower under anaerobic conditions than under aerobic conditions. Note that the ATP forming efficiency is a little low in practice, and thus 1.5–2.5 moles of ATP may be formed in practice from 1 mole of NADH. Likewise, the amount of ATP generation for $FADH_2$ is also less than 2 moles of ATP per NADH.

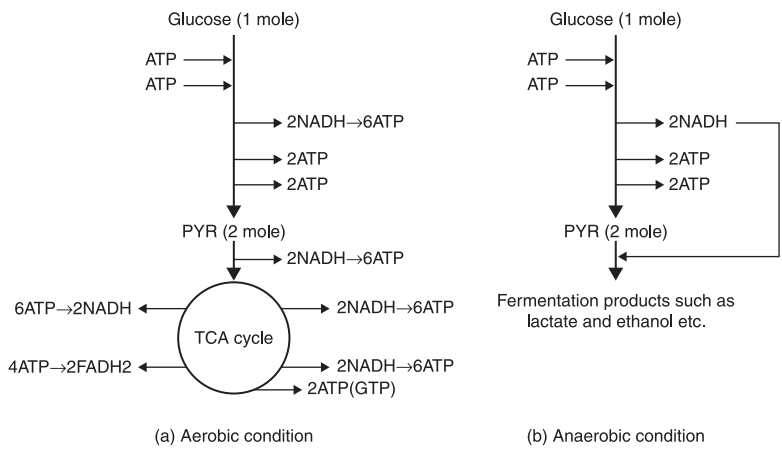


Figure 1.35 ATP balance for aerobic and anaerobic conditions

1.14 Metabolic regulation and futile cycle

Metabolic regulation of a cell achieves cell growth by optimizing ATP generation (catabolism) and cell synthesis (anabolism) during the cell

Published by Woodhead Publishing Limited, 2013

growth phase. Moreover, the cell regulates the metabolism to cope with various kinds of stresses caused by changes in the culture environment, and thus it is not easy to understand the whole metabolic regulation mechanism. Metabolic regulation occurs at both gene and enzyme levels, where enzyme level regulation is typically made by allosteric regulation, which is attained by changing the 3D structure by binding the specific metabolites, etc. For example, G6PDH and 6PGDH are inhibited by NADPH, while Pfk is inhibited by PEP. PDH is also inhibited by NADH, ATP, AcCoA, and so on. Consider the regulation of Pfk and Fbp in the EMP pathway, where Pfk catalyzes the following reaction:

$$\mathrm{F6p} + \mathrm{ATP} \xrightarrow{\mathrm{Pfk}} \mathrm{F1,6BP} + \mathrm{ADP} \tag{1.44}$$

while Fbp catalyzes the reverse reaction, such as:

$$\mathrm{F1,6BP} \xrightarrow{\mathrm{Fbp}} \mathrm{F6P} + \mathrm{P_i} \tag{1.45}$$

If these reactions occur at the same time, the energy generated by one pathway is used by the other pathway without efficient use. This phenomenon is called a **futile cycle**, where this occurs due to independent control by each pathway. A similar phenomenon can be seen for Ppc and Pck for anaplerotic and gluconeogenetic pathways, such as:

$$\mathrm{PEP} + \mathrm{CO_2} \xrightarrow{\mathrm{Ppc}} \mathrm{OAA} + \mathrm{ADP} \tag{1.46}$$

and

$$\mathrm{OAA} + \mathrm{ADP} \xrightarrow{\mathrm{Pck}} \mathrm{PEP} + \mathrm{ATP} \tag{1.47}$$

Note that these occur depending on the culture conditions.

1.15 References

Atkinson, D.E. (1968) 'The energy charge of the adenylate pool as a regulatory parameter: Interaction with feedback modifiers', *Biochemistry*, 7: 43030–4.

Bekker, M., de Vries, S., Ter Beek, A., Hellingwerf, K.J., and Teixeira de Mattos, M.J. (2009) 'Respiration of *Escherichia coli* can be fully uncoupled via the non-electrogenic terminal cytochrome *bd*- II oxidase', *Journal of Bacteriology*, 191: 5510–17.

Deutscher, J., Franke, C., and Postma, P.W. (2006) 'How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria', *Microbiology and Molecular Biology Reviews*, 70(4): 939–1031.

Doelle, H.W. (1975) *Bacterial metabolism*, 2nd edition. New York: Academic Press.

Gennis, R.B. and Stewart, V. (1996) 'Respiration', in: Escherichia coli *and* Salmonella: *Cellular and Molecular Biology*, 2nd edition, edited by F.C. Neidhardt et al. Washington, DC: American Society for Microbiology. pp. 217–61.

Gibson, F. and Cox, G.B. (1993) 'The use of mutants of *Escherichia coli* K12 in studying electron transport and oxidative phosphorylation', *Essays in Biochemistry*, 9: 1–29.

Hausman, S.Z., Thompson, J., and London, J. (1984) 'Futile xylitol cycle in *Lactobacillus casei*,' *Journal of Bacteriology*, 160: 211–15.

Horton, H.R., Morgan, L.A., Ochs, R.S., Rawn, J.D., and Serimgeour, K.G. (1996) *Principles of Biochemistry*. Prentice Hall Inc.

Ingledew, W.J., Ohnishi, T., and Salerno, J.C. (1995) 'Studies on a stabilization of ubisemiquinone by *Escherichia coli*. Quinol oxidase, cytochrome bo', *European Journal of Biochemistry*, 227: 903–8.

Koebman, B.J., Westerhoff, H.V., Snoep, J.L., Nilsson, O., and Jensen, P.R. (2002) 'The glycolytic flux in *E. coli* is controlled by the demand for ATP', *Journal of Bacteriology*, 184: 3909–16.

Kornberg, H.L. (2001) 'Routes for fructose utilization by *Escherichia coli*', *Journal of Molecular Microbiology and Biotechnology*, 3: 355–9.

Lehninger, A.L., Nelson, D.L., and Cox, M.C. (1993) *Principles of Biochemistry*. New York: Worth Publishers.

Mason, M.G., Shepherd, M., Nicholls, P., Dobbin, P.S. et al. (2009) 'Cytochrome *bd* confers nitric oxide resistance to *Escherichia coli*', *Nature Chemical Biology*, 5: 94–6.

Mobious, K., Arias-Cartin, R., Breckau, D. et al. (2010) 'Heme biosynthesis is coupled to electron transport chains for energy generation', *Proceedings of the National Academy of Sciences of USA*, 107(23): 10436–41.

Saier, M.H. (2000) 'Vectorial metabolism and evolution of transport systems', *Journal of Bacteriology*, 182: 5029–35.

Sato-Watanabe, M., Mogi, T., Miyoshi, H., and Anraku, Y. (1998) 'Characterization and functional role of the QH site of *bo*-type ubiquinol oxidase from *Escherichia coli*', *Biochemistry*, 37: 5356–61.

Soballe, B. and Poole, R.K. (1999) 'Microbial ubiquinones: Multiple roles in respiration, gene regulation and oxidative stress management', *Microbiology*, 145: 1817–30.

Stryer, L. (1995) *Biochemistry*, 4th edition. New York: W.H. Freeman and Company.

Voet, D. and Voet, J.G. (1995) *Biochemistry*, 2nd edition. New York: John Wiley & Sons.

Webb, E.C. (1992) *Enzyme nomenclature*. Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology. New York: Academic Press.

Published by Woodhead Publishing Limited, 2013