Department of biology

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((Microbial Physiology))

Stage (-3-)

LEC- ((6))

energy production in the microbial cells

By

Asst.Lec. Dhuha .S Al-khafaji

Lec.6: Chemical reaction for energy production in the microbial cells :

Glycolysis

Glucose is metabolized through the Embden–Meyerhof–Parnas (EMP) pathway and hexose monophosphate (HMP) pathway and the metabolic product, pyruvate, is decarboxylated oxidatively to acetyl-CoA to be oxidized through the tricarboxylic acid (TCA) cycle. Twelve intermediates of these pathways are used as carbon skeletons for biosynthesis , Heterotrophs that utilize organic compounds other than carbohydrates convert their substrates into one or more of these , glucose metabolism through glycolysis intermediates. For this reason and the TCA cycle is called central metabolism .

EMP pathway

Many anaerobic and enteric bacteria transport glucose via group translocation (phosphotransferase system, PTS) in the form of glucose-6-phosphate. Glucose transported through active transport is phosphorylated by hexokinase:

$$\text{glucose} + \text{ATP} \xrightarrow{\text{hexokinase}} \text{glucose-6-phosphate} + \text{ADP}$$

Hexokinase can phosphorylate other hexoses such as mannose, and requires Mg^{2} for activity. The enzyme cannot catalyze the reverse reaction. Glucose-6-phosphate can also be obtained from glycogen :

$$[\text{glucose}]n + \text{Pi} \xrightarrow{\text{phosphorylase}} [\text{glucose}]n\text{–}1 + \text{glucose-1-phosphate}$$

(glycogen) (glycogen)

$$\text{glucose-1-phosphate} \xrightleftharpoons{\text{phosphoglucomutase}} \text{glucose-6-phosphate}$$

Glucose-6-phosphate is a precursor for the biosynthesis of polysaccharides as well as a substrate of the EMP pathway , which is the commonest glycolytic pathway in all kinds of organisms.

1. Phosphofructokinase (PFK): key enzyme of the EMP pathway

Glucose-6-phosphate is isomerized to fructose-6-phosphate before being phosphorylated to fructose-1,6-diphosphate by the action of phosphofructokinase (PFK) . These two reactions require Mg^2 . Glucose-6-phosphate isomerase catalyzes the reverse reaction, but phosphofructokinase does not. The irreversibility of an enzyme is due to thermodynamic reasons, and many enzymes that do not catalyze the reversible reaction are regulated. PFK is the key enzyme of the EMP pathway. If this enzyme is present in a given prokaryote, it can be assumed that this organism catabolizes glucose through the EMP pathway. Fructose-6-phosphate is the precursor of amino sugars and their polymers such as murein . Fructose-1,6-diphosphate aldolase cleaves fructose-1,6-diphosphate to two molecules of triose-phosphate. This aldolase catalyzes the reverse reaction, and participates in gluconeogenesis , producing hexose-phosphate when non-carbohydrate substrates are used as carbon sources.

2. ATP synthesis and production of pyruvate

Triose-phosphate isomerase equilibrates dihydroxyacetone phosphate and glyceraldehyde-3-phosphate produced from fructose- 1,6-diphosphate. Phospholipids are synthesized from glyceraldehyde-3-phosphate . Glyceraldehyde-3-phosphate is oxidized to 1,3-diphosphoglycerate by glyceraldehyde-3-phosphate dehydrogenase. This endergonic reaction is efficiently pulled by the following exergonic reaction catalyzed by 3-phosphoglycerate kinase . This enzyme requires

Mg^2, as do most kinases, and ATP generation in this reaction is an example of substrate-level-phosphorylation. 3-phosphoglycerate is the starting material for the synthesis of amino acids, serine, glycine and cysteine

3-phosphoglycerate kinase

1,3-diphosphoglycerate + ADP ⟷ 3-phosphoglycerate + ATP

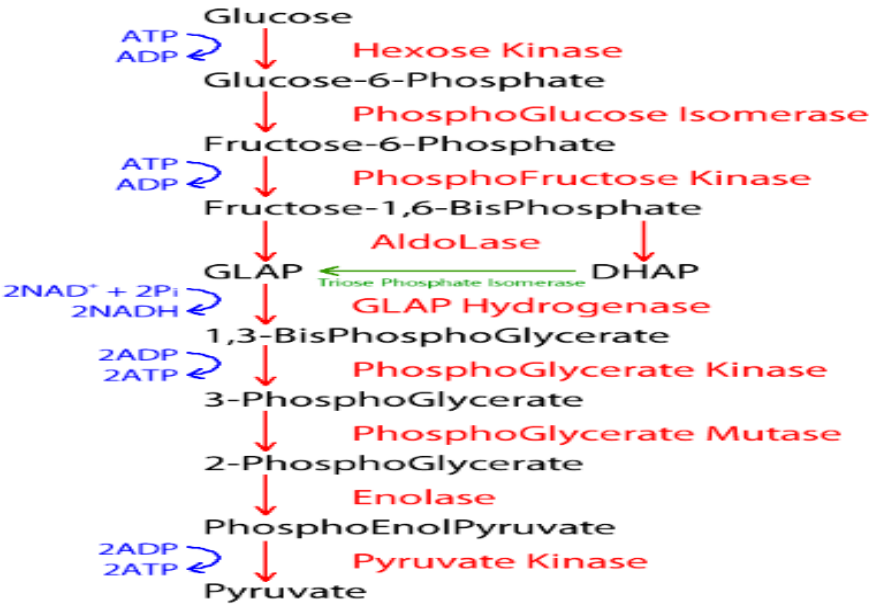
3-phosphoglycerate is converted to 2-phosphoglycerate by phosphoglycerate mutase which requires 2,3-diphosphoglycerate as a coenzyme . 2-phosphoglycerate is dehydrated to phosphoenolpyruvate (PEP) by an enolase in the presence of divalent cations such as Mg^2 and Mn^2. PEP is used to generate ATP with the reaction of the last enzyme (1 figure) in the EMP pathway in the presence of Mg^2 and K^1 .

pyruvate kinase

phosphoenolpyruvate + ADP ⟶ pyruvate + ATP

PEP supplies energy in group translocation, and is used to synthesize aromatic amino acids . Glyceraldehyde-3-phosphate is an intermediate in the HMP and ED pathways and the reactions from this triose-phosphate are shared with both these pathways. Four ATPs are synthesized and two high energy phosphate bonds are consumed in this pathway, resulting in a net gain of two ATPs per glucose oxidized. The NADH reduced in the glycolytic pathway is reoxidized in aerobic and anaerobic respiration , and in fermentation , reducing various electron acceptors depending on the organism and on their availability.

Figure



He

xo

se

monophosphate (HMP) pathway

When Escherichia coli grows on glucose as the sole carbon and energy source, about 72% of the substrate is metabolized through the EMP pathway, and the HMP pathway consumes the remaining 28%. This is because the EMP cannot meet all the requirements for biosynthesis. The HMP pathway provides the biosynthetic metabolism with pentose- 5-phosphate, erythrose-4-phosphate and NADPH. This pathway is also called the pentose phosphate pathway. NADPH is used to supply reducing power in biosynthetic processes. $NADP^+$ is reduced only by isocitrate dehydrogenase , when glucose is metabolized through the EMP pathway and TCA cycle. NADH is seldom used in biosynthetic reactions. Most of the NADPH needed for biosynthesis arises from the HMP pathway. The HMP pathway can be discussed in **three steps** . During the **initial step** of the HMP pathway, glucose-6-phosphate is oxidized to ribulose-5-phosphate and CO2, reducing $NADP^+$. Glucose-6-phosphate dehydrogenase, lactonase and 6-phosphogluconate dehydrogenase catalyze these reactions. In the **following reactions**, ribulose-5-phosphate is converted to ribose- 5-phosphate and xylulose-5-phosphate by the

action of isomerase and epimerase. **Finally,** the pentose-5-phosphates are transformed to glucose-6-phosphate and glyceraldehyde-3-phosphate through carbon rearrangement by transaldolase and transketolase. A transaldolase transfers a 3-carbon fragment, and a 2-carbon fragment transfer is catalyzed by a transketolase. HMP can be summarized as:

glucose-6-phosphate + $2NADP^+$ ⟶ glyceraldehyde-3-phosphate + 3CO2 + 2NADPH + 2H+

Ribose-5-phosphate is the precursor for nucleotide synthesis, and aromatic amino acids are produced from erythrose-4-phosphate. NADPH supplies reducing power during biosynthesis . Some eukaryotic microorganisms metabolize more glucose through the HMP pathway when they use nitrate as their nitrogen source. They use NADPH in assimilatory nitrate reduction .

Additional functions of the HMP pathway

In addition to supplying precursors and reducing power for biosynthesis from glucose, the HMP and related pathways have some other functions. HMP is the major glycolytic metabolism in microbes that (1) utilize pentoses, and (2) do not possess other glycolytic activities. The HMP cycle is also employed for the complete oxidation of sugars in bacteria lacking a functional TCA cycle.

1 . Utilization of pentoses

Pentoses are phosphorylated and metabolized to fructose- 6-phosphate and glyceraldehyde-3-phosphate through steps 2 and 3 of theHMP pathway.

2 . Oxidative HMP cycle

Thiobacillus novellus and Brucella abortus oxidize glucose completely although they lack a functional EMP or ED pathway. Glucose is oxidized through the oxidative HMP cycle . Glyceraldehyde- 3-phosphate is oxidized to pyruvate as in the EMP pathway. The HMP cycle is found in species of Gluconobacter which do not have a functional TCA cycle. These bacteria possess the incomplete TCA fork to meet the supply of biosynthetic precursors .