

MICROBIAL BIOCHEMISTRY
3rd Year Licence — Biotechnology and health

Carbohydrate Catabolism

What you will learn

- Name the three pathways that convert glucose into pyruvate and say in which organisms each one occurs.
- Describe the four stages of glycolysis and calculate its ATP balance.
- Explain the two stages of the pentose phosphate pathway and why it matters for biosynthesis.
- Describe the Entner-Doudoroff (KDPG) pathway.
- List the four ways in which pyruvate is oxidised or decarboxylated.

1. Overview: three routes from glucose to pyruvate

Pyruvate, a three-carbon (C3) compound, is one of the most important intermediates of metabolism. Cells can obtain it from glucose by three different routes (Figure 1):

- **Glycolysis**, also called the Embden-Meyerhof-Parnas (EMP) or fructose bisphosphate pathway, is the most common route.
- **The pentose phosphate pathway (PPP)**, also called the hexose monophosphate shunt, phosphogluconate pathway or Warburg-Dickens-Horecker pathway, occurs in many living organisms.
- **The Entner-Doudoroff pathway**, also called the KDPG pathway (2-keto-3-deoxy-6-phosphogluconate), is found only in some Gram-negative bacteria such as *Pseudomonas*, *Rhizobium* and *Agrobacterium*.

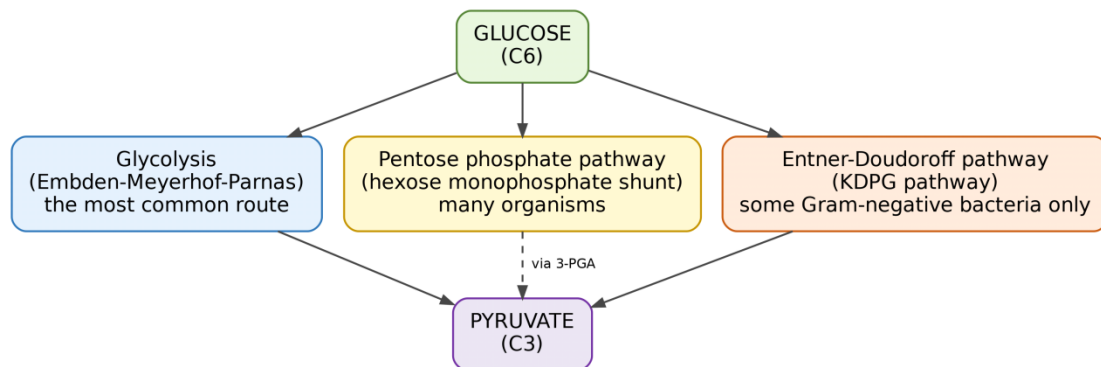


Figure 1. The three pathways that convert glucose into pyruvate.

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Table 1 shows how much each pathway contributes to hexose catabolism in a few microorganisms. Glycolysis dominates in most of them (*E. coli*, *B. subtilis*, *Penicillium*), whereas the KDPG pathway dominates in *Pseudomonas aeruginosa*, *Alcaligenes eutrophus* and *Gluconobacter oxydans*.

Table 1. Contribution of each pathway to hexose catabolism (%) in some microorganisms

Species	Glycolysis (%)	Pentose phosphate pathway (%)	KDPG pathway (%)
<i>Candida utilis</i>	70-80	30-20	-
<i>Streptomyces griseus</i>	97	3	-
<i>Penicillium chrysogenum</i>	77	-	-
<i>Escherichia coli</i>	72	-	-
<i>Bacillus subtilis</i>	74	-	-
<i>Pseudomonas aeruginosa</i>	23	-	71
<i>Gluconobacter oxydans</i>	-	-	100
<i>Pseudomonas saccharophila</i>	28	26	46
<i>Alcaligenes eutrophus</i>	29	-	71

2. Glycolysis (EMP pathway)

Glycolysis does not require oxygen. It is made of **ten reactions** grouped into **four stages**: activation, cleavage, oxidation and ATP generation (Figure 2).

Stage 1: Activation (reactions 1-3)

Glucose is phosphorylated. The first ATP is used to form glucose-6-phosphate (G6P); two further reactions convert G6P into fructose-1,6-bisphosphate, and a second ATP is consumed in the third reaction. **Cost: 2 ATP.**

Stage 2: Cleavage (reactions 4-5)

Fructose-1,6-bisphosphate is split into two three-carbon compounds: glyceraldehyde-3-phosphate (3-PGA) and dihydroxyacetone phosphate (DHAP). DHAP is then converted into a second molecule of 3-PGA, so **one glucose gives two 3-PGA.**

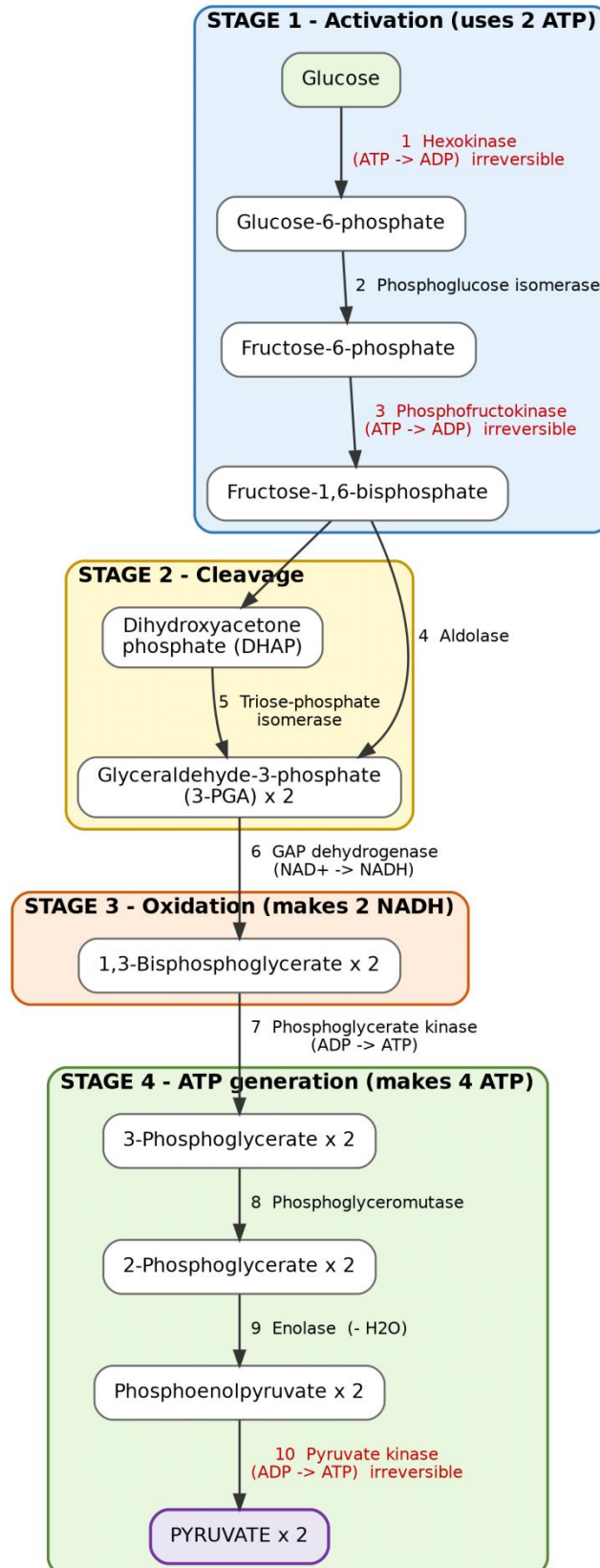
Stage 3: Oxidation (reaction 6)

Two electrons and two protons are removed from 3-PGA. The electrons and one proton are captured by NAD^+ to form **NADH**; the second proton is released into the cell. The oxidation produces a high-energy phosphorylated compound (1,3-bisphosphoglycerate) that is used for ATP generation in the next stage.

Stage 4: ATP generation (reactions 7-10)

Four reactions convert this compound into pyruvate and produce ATP. Because each glucose gives two 3-PGA molecules, this stage yields 4 ATP per glucose.

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Figure 2. The ten reactions of glycolysis, grouped into four stages (irreversible steps in red).

ATP balance

Stage	Description	ATP balance
Stage 1	Activation (ATP consumed)	-2 ATP
Stage 4	ATP generation	+4 ATP
Net result		+2 ATP per glucose

Key points

- Net yield: **2 ATP** (and 2 NADH) per glucose.
- All reactions are reversible **except three**, catalysed by **hexokinase**, **phosphofructokinase** and **pyruvate kinase**.
- Glycolysis is not very efficient in energy terms, but it supplies the intermediates needed for biosynthesis in prokaryotes and eukaryotes.
- During fermentation it is the **main source of ATP**.

3. The pentose phosphate pathway (PPP)

The PPP has **two stages**: an oxidative stage that starts from glucose-6-phosphate, and an interconversion stage in which pentose phosphates are converted into hexose phosphates and vice versa. Together they form a metabolic cycle (Figure 3).

Oxidative stage

G6P is dehydrogenated to 6-phosphogluconolactone, producing a first NADPH. The lactone is hydrolysed to 6-phosphogluconate, which undergoes oxidative decarboxylation (second NADPH, one CO₂ released) to give ribulose-5-phosphate.

Interconversion stage

Ribulose-5-phosphate is in equilibrium with ribose-5-phosphate and xylulose-5-phosphate. The enzymes **transketolase** and **transaldolase** transform these pentose phosphates into fructose-6-phosphate (F6P) and 3-PGA.

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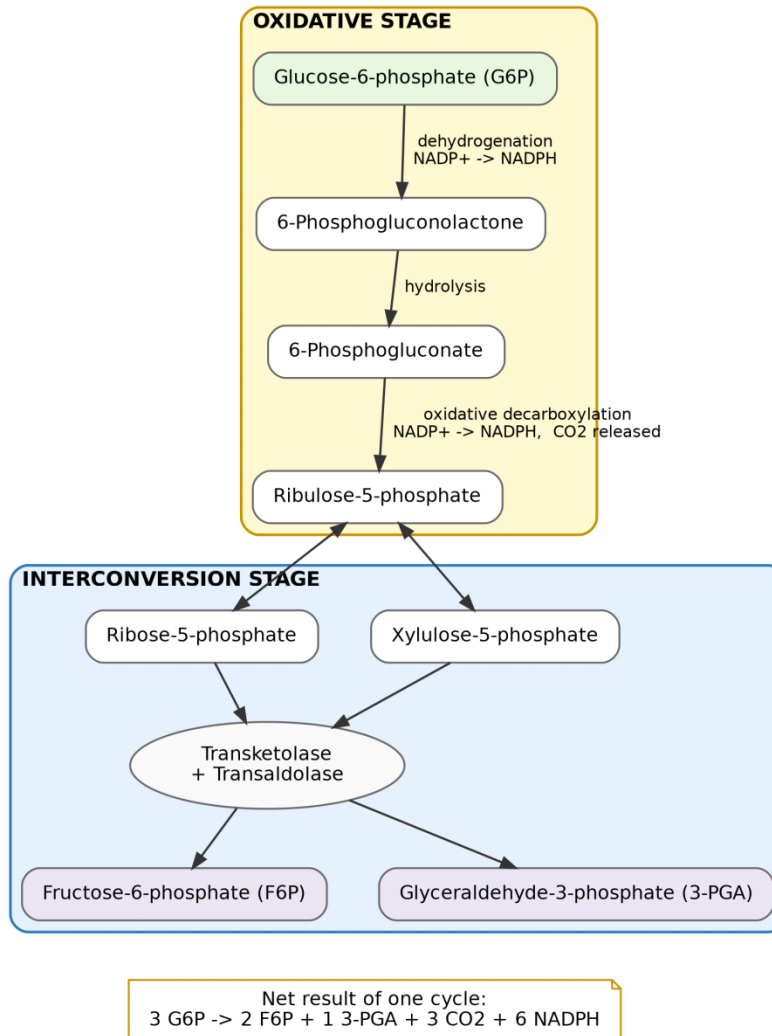


Figure 3. The pentose phosphate pathway.

Net result of one cycle: $3 \text{ G6P} \rightarrow 2 \text{ F6P} + 1 \text{ 3-PGA} + 3 \text{ CO}_2 + 6 \text{ NADPH}$.

Metabolic role

The PPP is a secondary pathway to glycolysis. Its main functions are to:

- produce **NADPH**, needed for many biosynthetic reactions;
- supply precursors: pentose phosphates, erythrose phosphate and 3-PGA.

Some of these products have specific uses:

- **ribose-5-phosphate** is essential for the synthesis of nucleotides and nucleic acids;
- **ribulose monophosphate** takes part in the formaldehyde fixation cycle of methylotrophic bacteria;
- **ribulose diphosphate** is central to the CO_2 fixation cycle (Calvin cycle).

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4. The Entner-Doudoroff (KDPG) pathway

The first step is the same as in the PPP: G6P is dehydrogenated to 6-phosphogluconate. Loss of a water molecule then gives **2-keto-3-deoxy-6-phosphogluconate (KDPG)**, which a specific aldolase cleaves into **pyruvate** and **3-PGA**. The 3-PGA is in turn oxidised to pyruvate by the lower part of glycolysis (Figure 4).

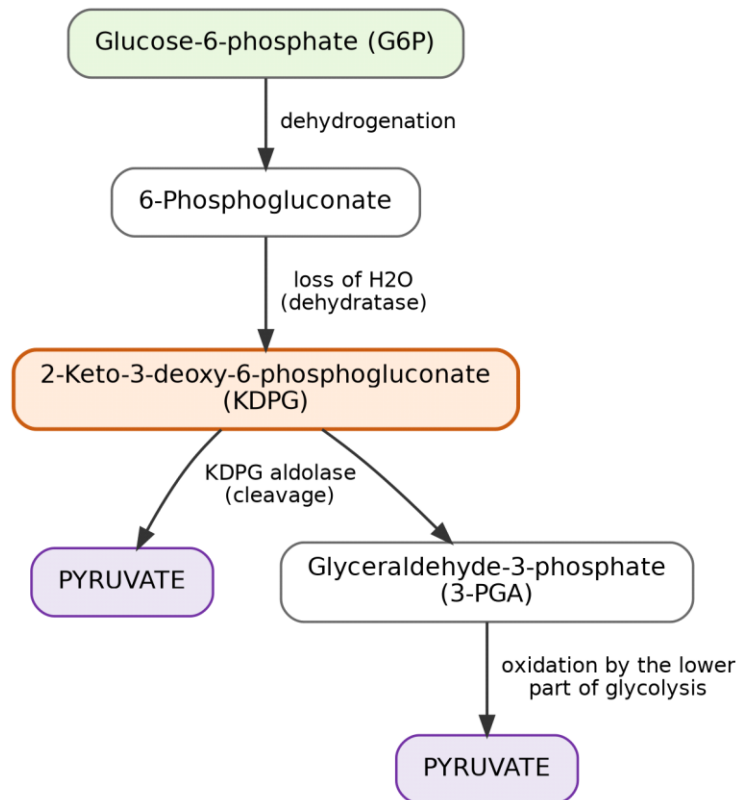


Figure 4. The Entner-Doudoroff pathway in Gram-negative bacteria.

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Table 2. Reactions of pyruvate

No.	Reaction	Enzyme	Where it occurs
1	pyruvate + CoA + NAD ⁺ → acetyl-CoA + NADH + H ⁺ + CO ₂	Pyruvate dehydrogenase (multienzyme complex)	All aerobic organisms; acetyl-CoA then enters the Krebs cycle
2	pyruvate + CoA + 2 Fd ⁻ → acetyl-CoA + 2 FdH + CO ₂	Pyruvate:ferredoxin oxidoreductase	Many anaerobic bacteria, e.g. Clostridia
3	pyruvate + CoA → acetyl-CoA + formate	Pyruvate:formate lyase	Several anaerobic bacteria (especially Enterobacteriaceae) and phototrophic organisms
4	pyruvate → acetaldehyde + CO ₂ (acetaldehyde is then reduced to ethanol)	Pyruvate decarboxylase	Yeasts and some bacteria that excrete ethanol

6. Summary: comparing the three pathways

	Glycolysis (EMP)	Pentose phosphate	Entner-Doudoroff (KDPG)
Starting point	Glucose	G6P	G6P
Key intermediate	Fructose-1,6-bisphosphate	Ribulose-5-phosphate	KDPG
Main products	Pyruvate, 2 ATP (net), 2 NADH	NADPH, pentose phosphates, 3-PGA, F6P, CO ₂	Pyruvate + 3-PGA (then pyruvate)
Occurrence	Most common route, prokaryotes and eukaryotes	Many organisms (secondary pathway)	Some Gram-negative bacteria only
Main role	ATP, biosynthetic intermediates	NADPH and precursors for biosynthesis	Alternative route to pyruvate