Glycolysis

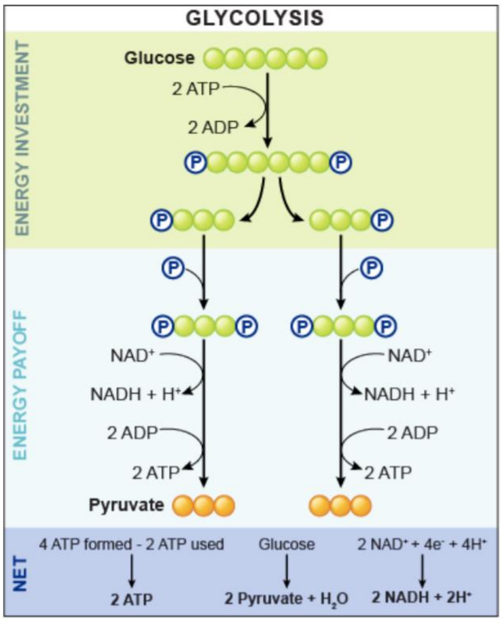
The metabolic pathway which **converts glucose into pyruvate**, producing **free energy and oxidation energy** which is used **to produce ATP and NADH.**

One of the principle pathways for **generation of ATP** in cells.

Occurs in all cells in the human body.

Glycolysis is not the only pathway for glucose- Glucose can take a range of pathways such as the pentose-phosphate pathway (for the generation of NADH), or storage by forming glycogen.

General Steps of Glycolysis



Glycolysis has two main stages:

- Energy Investment Steps
  - 2ATP is utilised to phosphorylate glucose
- Energy Payoff Stage
  - To make up for the investment of energy
  - For each of the two 3-carbon sugars
    - 2ADP is phosphorylated to 2ATP
    - 1NAD$^+$ is reduced to NADH + H$^+$
  - Involves **substrate level phosphorylation**
    - Generation of ATP by transfer of a substrate bound phosphate to ADP

Therefore, the **NET INCOME** for each glucose in the reaction is

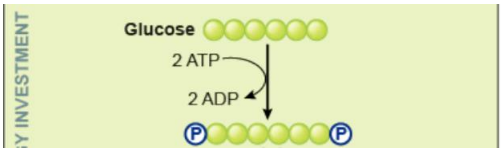
- **2ATP**
  - 2ATP invested and 4 produced
- **2 NADH + 2H$^+$**
  - 1NADH + H$^+$ produced for each of the two 3-carbon sugars
- And Glucose is converted **to 2 Pyruvate + H_2O** which can be used in later reactions

INVESTMENT STAGE

1. The Hexokinase Reaction – Phosphorylation of Glucose

Glucose is phosphorylated by hexokinase via the dephosphorylation of 1ATP → 1ADP, forming glucose 6-phosphate

← Note the hexokinase reaction only uses 1 ATP and makes a 6-carbon sugar with only one phosphate. The other ATP and phosphate come in on the next step.

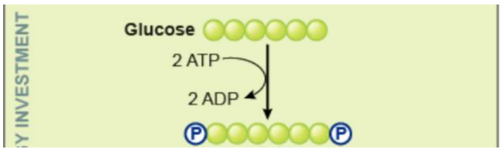


- **Substrate: Glucose + ATP**
- **Product: Glucose 6-Phosphate + ADP**
  - After the hexokinase reaction, glucose 6-phosphate can continue down the glycolysis pathway, ***or*** it can be used in the pentose phosphate pathway, it can be stored as glycogen, or it can enter a range of other pathways
- **Investment: ATP → ADP**
- Hexokinase Enzyme
  - Works by induced fit, meaning that the enzyme adapts to fit the substrate's transition state
- The hexokinase reaction works to **trap glucose within the cell**
  - The GLUT transporter brings glucose into the cell, **but** it also allows glucose to exit.
  - By converting glucose to glucose 6-phosphate, we are **preventing it from being able to exit the cell**
    - GLUT cannot transport glucose 6-phosphate

2. Isomerisation of Glucose 6-phosphate to Fructose 6-phosophate

3. Phosphofructokinase Reaction - Addition to phosphate to the other end of glucose 6-phosphate

Fructose 6-phosphate is phosphorylated by phosphofructokinase, via the dephosphorylation of 1ATP → 1ADP, forming fructose 1,6-bisphosphate (a 6-carbon molecule with a phosphate on each end)



← Note this reaction only uses 1 and adds only 1 phosphate. The other ATP and phosphate were respectively used and added in the hexokinase reaction.

- **Substrate: Glucose 6-phosphate + ATP**
- **Product: 6-carbon molecule with phosphates on each end + ADP**
- **Investment: ATP → ADP**

A REGULATED STEP!

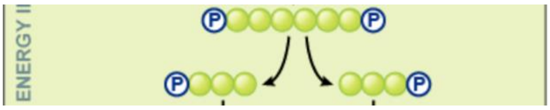
- Positively regulated by: **AMP and Fructose 2,6-bisphosphate**
- Negatively regulated by: **ATP**
- Therefore, proceeds when AMP levels are rising
  - AMP occurs when 2ADP are converted to ATP. Is a signal that the body needs more energy, therefore the glycolysis reaction needs to speed up
- At low concentrations of ATP, an increase in ATP will speed the reaction up.
  At high concentrations, ATP will begin acting as an allosteric inhibitor and will decrease the rate of reaction.
- Fructose 2,6-bisphosphate positively regulates glycolysis, but negatively regulates the reverse (gluco**neo**genesis)

4. The Aldolase Reaction- Splitting of the 6 carbon molecule (with 2 phosphates) into two 3 carbon molecules with 1 phosphate each.

The 6 carbon molecule is split into two 3 carbon molecules with one phosphate each

- **Substrate:** 6 carbon molecule with 2 phosphtaes
- **Product:** Two 3 carbon molecules with 1 phosphate each
- **No investment**

- Under standard conditions, this would be highly unfavourable. However, the use of ATP in the prior reactions offsets this, resulting in this reaction being at equilibrium under physiological conditions in the cell.



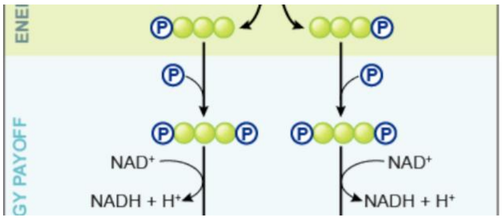
PAYOFF STAGE

<u>5. Glyceraldehyde-3-phosphate Dehydrogenase Reaction –</u> Phosphorylation of 3-carbon molecules, and production of oxidation energy for storage on NADH

Each of the 3 carbon molecules are phosphorylated, preparing them for the production of ATP in the phosphoglycerate kinase reaction.

The molecules are then oxidised, and the oxidation energy is captured in the form of 1NADH for each 3 carbon molecule.

- **Payoff: 1NADH per 3 carbon molecule**



<u>6. Phosphoglycerate Kinase Reaction (Substrate-Level Phosphorylation 1) –</u> 3 carbon molecules are dephosphorylated, and phosphates are transferred to ADP, forming ATP

1 ATP is generated for each of the carbon molecules by transfer of a substrate bound phosphate from each of the 3 carbon molecules to ADP

- **Payoff: 1 ATP per 3 carbon molecule**

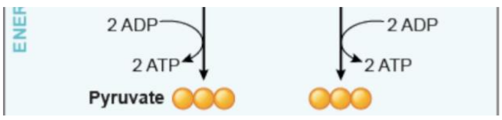
← Part 1

<u>7. Pyruvate Kinase Reaction (Substrate-Level Phosphorylation 2) –</u> The 3 carbon molecule is converted into phosphoenolpyruvate (PEP), which is then dephosphorylated into pyruvate. Phosphates are transferred to ADP, forming ATP

1 ATP is generated for each of the carbon molecules by transfer of a substrate bound phosphate from each of the 3 carbon molecules to ADP

At the end of this reaction, both of the 3 carbon molecules have been converted to **pyruvate.**

- **Payoff: 1 ATP per 3 carbon molecule**
- **Product: Pyruvate**



← Part 2

<u>A REGULATED STEP!</u>

- <u>Positively regulated by:</u> **Fructose 1,6-bisphosphate (product of phosphofructokinase reaction)**
- <u>Negatively regulated by:</u> **ATP**
- Therefore, proceeds when ATP levels are rising the reaction slows as it is not needed as much anymore
- When there is lots of product to convert, the reaction speeds up

Aerobic vs. Anaerobic Routes of Pyruvate after Glycolysis

Aerobic

- Pyruvate is transported into the mitochondria where it is oxidised in the TCA cycle which (*requires oxygen)*, producing oxidation-energy which is stored on NADH molecules.
- The NADH is then used in the electron transport chain to produce a **high amount of ATP.**
- *2ATP preserved from substrate-level phosphorylation*
  More ATP is produced via electron transport chain
  Requires Oxygen
  Takes place in the mitochondria

Anaerobic

- Using the NADH produced in earlier steps of glycolysis (the glyceralderhyde-3-phosphate dehydrogenase reaction), **pyruvate is converted to lactate**.
  - **Lactate may then be converted back to pyruvate** for aerobic metabolism or further anaerobic metabolism
- This does not produce any ATP or NADH, but **preserves the net 2ATP produced in glycolysis.**
- Done by human cells when there is not enough oxygen for aerobic metabolism (e.g. in muscles when there is not enough oxygen in the blood during exercise)
- *2ATP preserved from substrate-level phosphorylation*
  No extra ATP produced
  Pyruvate is converted to lactate which can be converted back to pyruvate

Regulation of Glycolysis

Two major allosteric enzyme regulatory steps

- The **Phosphofructokinase Reaction**
  - In the investment stage
  - Addition of an extra phosphate to frucose 6-phosphate, forming fructose 1,6-bisphosphate at the expense of ATP
  - **Upregulated by:** AMP
    - (Produced when 2ADP is being used to form ATP)
  - **Downregulated by:** ATP
    - At low concentrations of ATP, an increase in ATP will speed the reaction up.
      At high concentrations, ATP will begin acting as an allosteric inhibitor and will decrease the rate of reaction.
- The **Pyruvate Kinsase Reaction**
  - In the payoff stage
  - Substrate level phosphorylation 2: the 3 carbon molecule is converted to phosphoenolpyruvate (PEP) which is dephosphorylated into pyruvate. Transfers the phosphate from substrate to ADP, forming ATP.
  - **Upregulated by:** Fructose 1,6-bisphosphate (product of phosphofructokinase reaction- the other regulated step)
  - **Downregulated by:** ATP

Is also autoregulated by availability of ADP and NAD+

No ADP = no production of ATP

No NAD+ = no production of NADH