Life 20 - Glycolysis – Raven & Johnson Chapter 9 (parts)

Objectives

1: Know the location of glycolysis in a eukaryotic cell

2: Describe glycolysis in general terms, including the molecules that exist at its start and end and some intermediates

3: Know the total and net ATP production resulting from glycolysis

4: Explain what is meant by substrate-level phosphorylation

5: Understand the mechanisms that recycle NADH in anaerobic and aerobic conditions

6: Understand how proteins and fats are catabolised

Glycolysis ("breakdown of sugar")

Carbohydrate metabolism is channelled through glucose, a six carbon (6-C) sugar. Complete oxidation gives $\Delta G = - 3010 \text{ kJ mole}^{-1}$

Could be dismantled in many ways. Glycolysis has evolved as a sequence of 10 reactions that drive synthesis of ATP

Most easily viewed as two halves, each of 5 reactions

The first half primes, splits and rearranges the glucose molecule into two high-energy 3-C sugar phosphates

The second half extracts energy from these 3-C sugar phosphates to synthesise ATP

Followed by reactions that remove the end products so that further glycolysis can occur

The first half of glycolysis (Fig. 9.8, and simplified version)

Reaction (1) primes glucose with ATP to produce a high-energy sugar phosphate glucose 6-phosphate

The 6 refers to the carbon atom at which the phosphate is attached

Reaction (2) is a rearrangement to form another 6-C sugar phosphate

Reaction (3) is a second priming, attaching another high-energy phosphate from ATP to give fructose 1,6-biphosphate

Reaction (4) is a cleavage, into two 3-C sugar phosphates; one is glyceraldehyde 3-phosphate

The other cleavage product is converted into a second glyceraldehyde 3-phosphate in reaction (5) (by isomerase)

One 6-C glucose has thus been converted into two 3-C high-energy sugar phosphates

2 ATP molecules have been used in the process

The second half of glycolysis

5 more reactions that convert the 3-C sugar phosphates into pyruvate. Each reaction occurs twice per glucose molecule

Reaction (6) is an oxidation, with NAD^+ being reduced to NADH. 2 electrons are removed (with H)

The energy liberated also allows a high-energy phosphate (~P) bond to be produced from inorganic phosphate Pi

The result is a 3-C biphosphate molecule with two ~P groups,
1,3 biphosphoglycerate

One of these ~P is removed in reaction (7), to form 3-phosphoglycerate and synthesise ATP

The high-energy phosphate is transferred from 1,3 biphosphoglycerate to ADP to give ATP

This is substrate-level phosphorylation, when ~P is transferred directly to ADP from a substrate molecule (Fig. 9.5)

Reaction (8) is a rearrangement and reaction (9) is a dehydration, with a water molecule being removed

The other ~P is removed in reaction (10), to generate ATP; another substrate-level phosphorylation

All other steps in glycolysis have small ΔG and cannot form ATP

The final metabolic product of glycolysis is pyruvate. The net result is:

1 glucose + 2 NAD^+ + 2 Pi + 2 ADP $\Rightarrow$ 2 pyruvate + 2 ATP + 2 NADH + 2 H_2O

For each glucose, two ATP are consumed in priming, four ATP are produced by substrate-level phosphorylation, giving a net yield of 2 ATP

Two pyruvate molecules are also produced, and two NAD^+ are reduced to NADH

Associated reactions

The cellular supply of NAD^+ is limited, and a shortage of NAD^+ would soon stop glycolysis

To be sustainable, there must therefore be further reactions, where NADH is re-oxidised to NAD^+

There are three possibilities:

A: Fermentation. An organic molecule accepts the electron (and hydrogen)

Organic + NADH $\Rightarrow$ reduced organic + NAD^+

Bacteria carry out more than a dozen different types of fermentation, with different organic molecules as electron acceptors

The reduced molecule is usually an organic acid (acetic, butyric, lactic), or an alcohol (ethanol, butanol)

In muscle cells in anaerobic conditions (Fig. 9.24):

Pyruvate + NADH $\Rightarrow$ lactic acid + NAD^+

This causes fatigue as lactic acid accumulates, but regenerates NAD^+ in the short-term

In yeast (single celled fungi, eukaryote):

Pyruvate + NADH $\Rightarrow \Rightarrow$ Ethanol + CO_2 + NAD^+

Products seen as ethanol in brewing, rising in baking from CO_2. Proceeds until ethanol reaches 12% then yeast dies

Goldfish and carp also have fermentation to ethanol, unique in vertebrates as an important pathway

B: Anaerobic respiration. Respiration is where an inorganic molecule accepts the electron (and H)

This is found in some bacteria living in anaerobic conditions:

$CO_2 \Rightarrow CH_4$ in methanogens
$SO_4 \Rightarrow H_2S$ in sulphur bacteria

C: Aerobic respiration. The inorganic molecule is oxygen, a good electron acceptor

This occurs in some bacteria and in all eukaryotic cells in the presence of oxygen, inside the mitochondria

The first stage is the oxidation of pyruvate to acetyl-coenzyme A (acetyl-CoA)

Pyruvate + NAD^+ + CoA $\Rightarrow$ Acetyl-CoA + NADH + CO_2

This consumes a further NAD^+ to NADH, but eventually all the NADH gives its electrons (and H) to oxygen (Lecture 21)

Coenzyme A is a carrier of acetyl groups (2-C), as ATP carries energy/P and NADH carries electrons/H

Similar to NAD and ATP in having adenine, ribose and a pyrophosphate bridge. Plus pantothenic acid, a B-group vitamin

Acetyl-CoA is a hub of cellular metabolism. Almost all molecules catabolised for energy (carbohydrate, protein, lipid) pass through acetyl-CoA (Fig. 9.11)

When ATP is in short supply acetyl-CoA is used in energy production. When there is sufficient ATP, acetyl-CoA is used in fat synthesis to store energy

Biological significance of glycolysis

The energy harvested by glycolysis is 61 kJ $mole^{-1}$ glucose (2 ATP), compared to ΔG = -3010 kJ $mole^{-1}$ for complete oxidation with oxygen

The efficiency of glycolysis is therefore low, harvesting only about 2% of the total energy of the glucose molecules

But for what it does, the efficiency is high – about 31% of the free energy from glucose to lactate is harvested as ATP. It just does not carry oxidation very far; most of the energy is still unused in the products (lactate, ethanol etc)

For the first 1 billion years of life on earth, glycolysis was the primary means of generating ATP

This was among the earliest of biochemical pathways to evolve. Every existing organism is capable of glycolysis

No oxygen is involved - glycolysis can occur in anaerobic conditions

Takes place in the cytoplasm, with enzymes free in the solution, not bound to any membranes or organelles

All except the end molecules glucose & pyruvate (& lactate, ethanol etc) are phosphorylated, and cannot pass through cell membranes

They are therefore retained in the cytoplasm - another reason for these pathways to have been selected, kept in the cell

Metabolism of fats and proteins

Proteins are hydrolysed into amino acids by digestion, then deaminated in the cell; amino groups cleaved off to NH_3 (Fig. 9.22)

The resulting molecules are rearranged to acetyl-CoA. The energy yield of amino acids is similar to carbohydrates – they have a similar oxidation level (similar numbers of C-H bonds)

Ammonia is the basic nitrogenous waste product. It is excreted directly by most aquatic animals – crayfish. Or converted to urea (less toxic - in mammals), or uric acid (insoluble, excreted as solid to save water - in reptiles & birds)

Fats are catabolised by β-oxidation, in which 2-C acetyl groups are removed from the carbon chain (at the β position) (Fig. 9.23)

These acetyl groups enter metabolism as acetyl-CoA. E.g. the oxidation of palmitic acid, a 16-C fatty acid

First step is synthesis of the active form palmityl-CoA using ATP and CoA

$CH_3(CH_2)_{14}COOH$ +ATP +CoA $\Rightarrow$ $CH_3(CH_2)_{14}CO$-CoA

Then acetyl groups removed in a series of reactions:

$CH_3(CH_2)_{14}CO$-CoA + CoA $\Rightarrow\Rightarrow\Rightarrow\Rightarrow$ $CH_3(CH_2)_{12}CO$-CoA + acetyl-CoA
$\Rightarrow\Rightarrow\Rightarrow\Rightarrow$ $CH_3(CH_2)_{10}CO$-CoA + acetyl-CoA
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$\Rightarrow\Rightarrow\Rightarrow\Rightarrow$ $CH_3(CH_2)_4CO$-CoA + acetyl-CoA
$\Rightarrow\Rightarrow\Rightarrow\Rightarrow$ $CH_3(CH_2)_2CO$-CoA + acetyl-CoA
$\Rightarrow\Rightarrow\Rightarrow\Rightarrow$ CH_3CO-CoA + acetyl-CoA

Note the $(CH_2)_n$ where n decreases by 2 at each stage as an acetyl group is removed. In the final step CH_3CO-CoA (where n=0) is also acetyl-CoA

The 16-C fatty acid has been completely broken down into 8 acetyl-CoA, 8 two-carbon groups removed

Fats are more highly reduced than carbohydrates or proteins, as they contain many C-H bonds

They therefore have higher energy content, and are used for long-term energy storage