

8.3 Photosynthesis

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8.3.1 Light-dependent Reactions

Location of the Light-dependent Reactions

- Photosynthesis takes place in two distinct stages:
 - The light-dependent reaction, which relies on light directly
 - The light-independent reaction, which does not use light directly
- Both these reactions take place within the chloroplast
 - The light-dependent reaction takes place in the **thylakoid intermembrane space** and across the **thylakoid membrane**
 - **Thylakoids** are disc like structures which make up the grana in stacks of up to 100. They contain the photosynthesis pigment chlorophyll. Some may have tubular extensions (intergranal lamellae) which join up with thylakoids in adjacent grana
 - The **thylakoid membrane** contains a transfer chain where electrons are passed along a number of electron carriers in a series of oxidation-reduction reactions

Examiner Tip

The thylakoid intermembrane space is also referred to as the thylakoid lumen.





Products of the Light-dependent Reactions

- During the light-dependent reaction light energy is converted into chemical energy in the form of ATP and reduced NADP
- ATP and reduced NADP are produced from the **photolysis** of water by light energy:
 - Water is split into protons, electrons and oxygen
 - The protons are picked up by the hydrogen acceptor NADP+ thereby reducing it (NADPH, also called reduced NADP)
 - ATP is generated from the **phosphorylation** of ADP
- The useful products of the light-dependent reaction are transferred to the light-independent reaction within the chloroplast
- Oxygen is given off as a waste product of the light-dependent reaction



becomes reduced and can be written as NADPH. NADP can also be written as NADP+.

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Excitation of Electrons

- Chloroplasts contains the pigment chlorophyll, plus other accessory pigments
- These are grouped together as structures called **photosystems** which are located in the thylakoids
- Photosystems contain many chlorophyll molecules and a reaction centre
- Two types of photosystems exist:
 - Photosystem I contains the reaction centre P700 (as it is activated by a wavelength of light of 700nm)
 - Photosystem II contains the reaction centre P680 (as it is activated by a wavelength of light of 680nm)
- Chlorophyll molecules within Photosystem II absorb light energy, in the form of photons, and pass it to the reaction centre P680
- Electrons within the reaction centre of Photosystem II are then excited to a higher energy level by the photons of light
- The chlorophylls within the reaction centre are said to be **photoactivated**
- Excited electrons are able to be **donated** to an **electron acceptor** in a **reduction reaction**
 - In the light-dependent reaction the electron acceptor is called **plastoquinone**
 - Plastoquinone accepts two electrons from Photosystem II and is reduced
 - It then moves to another position in the thylakoid membrane
- This process is repeated with another plastoquinone molecule
- In total two plastoquinone molecules are reduced and four electrons are lost from the reaction centre



A photosystem used in the light-dependent reaction to excite electrons



Examiner Tip

Rather confusingly, the first photosystem to be activated in the light-dependent reaction is Photosystem II. Later in the reaction, Photosystem I is involved. This is because Photosystem I was the first to be discovered and therefore was named first.

Photolysis

- Photolysis occurs in Photosystem II during the light-dependent reaction of photosynthesis
- This occurs following the **reduction of plastoquinone** in Photosystem II:
 - The reaction centre acts as an **oxidising agent** and causes water molecules (that have been moved into the leaf by transport up the xylem vessels) to split during photolysis
- Water splits into protons, electrons and oxygen
 - The oxygen diffuses out of the leaf through stomata
 - The electrons are passed into the electron transport chain
 - The protons are picked up by the carrier molecules NADP forming reduced NADP
- The reaction can be summarised as $2H_2O \rightarrow O_2 + 4H^+ + 4e^-$
- The photolysis of water generates the electrons needed for:
 - Replacement of the electrons lost from the reaction centre in Photosystem II
 - Subsequent reactions of the light-dependent reaction



8.3.2 Photophosphorylation

The Electron Transport Chain in Photosynthesis

- Photophosphorylation is a process that uses light energy and the electron transport chain to generate ATP from ADP
 - Photo = light
 - Phosphorylation = addition of phosphate to ADP
- Photophosphorylation involves the following sequence of events:
 - 1. Light causes electrons in **photosystem II** to gain energy and become **excited**
 - The excitement of electrons can be referred to as photoactivation
 - 2. The excited electrons leave photosystem II and are passed down a series of **electron carriers** that form the **electron transport chain**
 - The electron transport chain occurs on the thylakoid membranes within the chloroplast
 - One of the electron carriers on the thylakoid membrane is known as plastoquinone; plastoquinone accepts a pair of electrons from photosystem II
 - 3. The electron carriers undergo a series of **redox reactions** as electrons are gained and lost from each carrier
 - Remember that:
 - Reduction = gain of electrons
 - Oxidation = loss of electrons
 - 4. As the electrons pass along the electron transport chain **energy is released**; this energy is used to **pump protons** across the thylakoid membrane, generating a **proton gradient** between the thylakoid space and the stroma (see below)
 - This proton gradient enables the phosphorylation of ADP to produce ATP during chemiosmosis (also below)
 - 5. The pair of electrons pass from the electron transport chain to **photosystem I**
 - 6. The electrons in photosystem I can also be excited by light energy, at which point they are used in the **reduction of NADP** (also covered in more detail below)
- The photophosphorylation process described here is referred to as **non-cyclic** as the electrons do not return to the location at which they started
- ATP and reduced NADP are the main products of photophosphorylation and are immediately passed to the light-independent reaction



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PHOTOACTIVATION - ELECTRONS IN THE PRIMARY PIGMENT MOLECULE OF EACH PHOTOSYSTEM ARE EXCITED TO A HIGHER ENERGY LEVEL





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ELECTRONS FROM THE PHOTOLYSIS OF WATER REPLACE THOSE LOST FROM PHOTOSYSTEM II

EXCITED ELECTRONS FROM PHOTOSYSTEM I AND HYDROGEN IONS FROM THE PHOTOLYSIS OF WATER BOTH COMBINE WITH NADP TO FORM REDUCED NADP

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Phosphorylation involves the electron transport chain and the production of ATP and reduced NADP



Remember that a redox reaction is one where reduction reactions (gain of electrons or hydrogen, loss of oxygen) and oxidation reactions (loss of electrons or hydrogen, gain of oxygen) happen together. This happens along the series of electron carriers in the thylakoid membrane as part of the electron transport chain.

Forming the Proton Gradient

- Electrons are passed from carrier to carrier in the electron transport chain
- As they do so they release energy which is used to pump protons from the stroma across the thylakoid membrane and into the thylakoid space
 - The thylakoid space is also known as the the thylakoid lumen
- The protons move via a proton pump
- A high concentration of protons builds inside the intermembrane space creating a concentration gradient
- Photolysis of water releases hydrogen ions which contribute to the proton gradient



Chemiosmosis in Photosynthesis

- The proton gradient within the intermembrane space of the thylakoid powers the synthesis of ATP
 - The protons travel down their concentration gradient through the membrane protein ATP synthase
 - Energy is released by the movement of protons and is used to make ATP from the phosphorylation of ADP
- This process is called **chemiosmosis**
- The ATP produced is used in the light-independent reaction





Reduction of NADP

- Photosystem I is involved in the reduction of NADP which is a key molecule used in the lightindependent reaction
 - Chlorophyll molecules in the reaction centre **absorb photons** of light energy
 - Electrons within the reaction centre are photoactivated to a higher energy level
 - They are passed to a **protein** on the outside of the thylakoid membrane called **ferredoxin** and reduce it
 - The reduced ferredoxin, along with protons that have passed through ATP synthase during chemiosmosis, are used to reduce NADP to NADPH (reduced NADP)
 - NADP + $2H^+$ + $2e^- \rightarrow NADPH$
 - The ferredoxin is now oxidised and free to be reused in this reaction again
 - Reduced NADP now carries a pair of electrons and can be passed into the light-independent reactions of photosynthesis





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8.3.3 Light-independent Reactions

Location of the Light-independent Reactions

- The light-independent reactions of photosynthesis take place in the stroma of the chloroplasts
- The stroma is within the double membrane and is a thick protein rich environment containing the **enzymes** needed for the light-independent reactions

Carbon Fixation

- The light-independent reactions of photosynthesis are also known as the **Calvin cycle**
- There are three main steps within the Calvin cycle:
 - 1. **Carbon fixation**: The enzyme **rubisco** catalyses the **fixation of carbon dioxide** by combination with a molecule of ribulose bisphosphate (RuBP), a 5C compound, to yield two molecules of glycerate 3-phosphate (GP), a 3C compound
 - 2. **Reduction: GP is reduced** to triose phosphate (TP) in a reaction involving reduced NADP and ATP 3. **Regeneration: RuBP is regenerated** from TP in reactions that use ATP
- Carbon dioxide is converted into carbohydrates, namely glucose, during the cycle in a series of anabolic reactions
 - Anabolic reactions require energy in order to build large complex molecules from smaller simpler ones
- The Calvin cycle relies on the products of the light-dependent reactions namely ATP and reduced NADP
- During the cycle **endergonic** reactions take place that involve the hydrolysis of ATP and oxidation of reduced NADP
- An **endergonic reaction** requires energy to be absorbed before the reaction can proceed **Carbon fixation details**
- Carbon dioxide is the source of carbon for all organisms that carry out photosynthesis
- Carbon fixation involves carbon dioxide (1C) being removed from the external environment and becoming part of the plant, and is then said to be "fixed"
 - It is transformed into a three-carbon compound (3C) called glycerate-3-phosphate (sometimes shortened to as GP)
- During the fixation step of the Calvin cycle carbon dioxide is combined with a five-carbon compound (5C) called ribulose bisphosphate (RuBP) to make an unstable six-carbon (6C) compound that splits into two molecules of glycerate-3-phosphate
- This reaction is catalysed by the enzyme rubisco
- Glycerate-3-phosphate is then used in the next step of the cycle



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Role of Reduced NADP and ATP

- Energy from ATP and hydrogen from reduced NADP (from the light-dependent reactions) are used to reduce glycerate-3-phosphate to a phosphorylated three-carbon molecule called triose phosphate (sometimes shortened to TP)
- After the reduction step **one sixth** of the triose phosphate is converted into **usable products** for the plant:
 - Hexose phosphates which can be used to produce carbohydrates such starch, sucrose or cellulose
 - Glycerol and fatty acids which join to form cell membranes
 - Production of **amino acids** for protein synthesis
- It is important that not all the triose phosphate is converted to alternative compounds for the plant, or the supplies of ribulose bisphosphate would run out
- The remaining triose phosphate is used to regenerate RuBP

💽 Examiner Tip

For the Calvin cycle to continue it needs a constant supply of RuBP and carbon dioxide. As much RuBP must be produced as is consumed. If three RuBP molecules are used then this generates six triose phosphates. Five of the triose phosphate molecules are needed to regenerate the three RuBPs molecules. So there would only be one left over to convert into other usable molecules for the plant (such as starch). To produce just one molecule of glucose, six turns of the Calvin cycle are needed.



Regeneration of RuBP

- The remaining five sixths of triose phosphate are used to **regenerate** the four-carbon compound ribulose bisphosphate **(RuBP)**
- This process requires **ATP** (from the light-dependent reaction)
- Once RuBP been has regenerated it can go on to fix further carbon dioxide and the cycle can begin again



Your notes

8.3.4 Investigating Carbon Fixation in Photosynthesis

Investigating Carbon Fixation in Photosynthesis

NOS: Developments in scientific research follow improvements in apparatus: sources of ¹⁴C and autoradiography enabled Calvin to elucidate the pathways of carbon fixation

- The Calvin cycle was named after American biochemist **Melvin Calvin** for his work in mapping the complete conversion of carbon dioxide to glucose
- The techniques used at the time were novel and showed developments in scientific research
- Calvin developed methods for growing algae in an apparatus he named "the lollipop" due to its shape
- This apparatus enabled Calvin to introduce radioactive carbon dioxide to the algae in order to study photosynthesis
- He also used paper chromatography and production of x-ray chromatograms to enable Calvin to identify compounds using in reactions during photosynthesis
- His approaches and methods were novel at the time and were only possible because of advancements in apparatus and technologies
- The experiments performed by Calvin show process of using radioactive carbon dioxide and autoradiography in explaining the reactions of the Calvin Cycle:
 - Radioactively labeled carbon-14 (¹⁴C) was introduced to the algae Chlorella in an apparatus called a lollipop (the experiments are sometimes referred to as the "lollipop experiment" due to the shape of the apparatus)
 - Light was shone on the lollipop vessel containing the *Chlorella* to induce photosynthesis and carbon-14 was incorporated into the algae
 - After varying time periods the **algae was killed** by heated alcohol which denatures proteins and enzymes within the cells and stops metabolic processes
 - The **pathway** of the radioactive carbon was **mapped and analysed** throughout the algae using two-dimensional **paper chromatography**
 - Chromatography **separated** out the different carbon compounds that had been made by the algae
 - Any radioactive carbon-14 atoms (that had been incorporated into either intermediates or products of photosynthesis) were identified using autoradiography (x-ray)
 - By **comparing** the different **time periods** in which the carbon compounds formed Calvin was able to map the order in which they were generated
- The results of the experiments showed that carbon was converted to carbohydrates during the lightindependent reactions of photosynthesis
- Today, the method Calvin used is called "feeding experiments"

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Your notes

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8.3.5 Chloroplast

Chloroplast Structure & Function

Structure

- Chloroplasts are the organelles in plant cells where **photosynthesis** occurs
- These organelles are roughly 2 10 μm in diameter (they are larger than mitochondria)
- Each chloroplast is surrounded by a **double-membrane envelope**
 - Each of the envelope membranes is a phospholipid bilayer
 - The **outer membrane** is permeable to a range of ions and small molecules
 - The **inner membrane** contains transport proteins that only allow certain molecules or ions to enter or leave the chloroplast
- Chloroplasts are filled with a cytosol-like fluid known as the **stroma**
 - CO₂, sugars, enzymes and other molecules are dissolved in the stroma
 - If the chloroplast has been photosynthesising there may be **starch grains** or **lipid droplets** in the stroma
- A separate system of membranes is found in the stroma
- This membrane system consists of a series of flattened fluid-filled sacs known as thylakoids
 - The thylakoid membranes contain pigments, enzymes and electron carriers
 - These thylakoids stack up to form structures known as grana (singular granum)
 - Grana are connected by membranous channels called **stroma lamellae**, which ensure the stacks of sacs are connected but distanced from each other



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Your notes



• Each pigment molecule passes energy down to the next pigment molecule in the cluster until it reaches the primary pigment reaction centre

Adaptations of chloroplasts to photosynthesis

- Stroma:
 - The gel-like fluid contains **enzymes** that catalyse the reactions of the light-independent stage
 - The stroma surrounds the grana and membranes, making the **transport** of products from the lightdependent stage into the stroma **rapid**
- Grana:
 - The granal stacks create a **large surface area** for the presence of many photosystems which allows for the **maximum** absorption of light
 - It also provides **more membrane space** for electron carriers and ATP synthase enzymes
- DNA:
 - The chloroplast DNA contains genes that code for some of the proteins and enzymes used in photosynthesis
- Ribosomes:

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- The presence of ribosomes allows for the **translation of proteins** coded by the chloroplast DNA
- Inner membrane of chloroplast envelope:
 - The selective transport proteins present in the inner membrane **control** the flow of molecules between the stroma and cytosol (the cytoplasm of the plant cell)
- Thylakoid space:
 - This is where a **proton gradient develops** (to generate ATP)
 - The space has a very small volume so a proton gradient can develop very quickly



8.3.6 Skills: Photosynthesis

Skills: Annotating Chloroplasts

- Electron micrographs may **differ** in shape and size depending on where the cross section of the organelle was taken
- Usually the following features should be notable and visible:
 - Round in shape
 - A double membrane exterior
 - Flattened discs, the **thylakoids**, arranged in stacks, the grana, **connected by** thin tubes, the **lamellae**
 - Ribosomes and DNA are not usually visible
 - Starch granules may be visible as dark spots within the stroma



ABSORPTION OF LIGHT

STROMA - CONTAINS ENZYMES FOR CALVIN CYCLE

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