

# Photosynthesis

# Contents

- \* The Process of Photosynthesis
- \* Separating Photosynthetic Pigments: Skills
- ✤ Absorption Spectra
- \* Absorption & Action Spectra: Skills
- \* Limiting Factors of Photosynthesis: Skills
- \* Carbon Dioxide Enrichment Experiments
- ✤ Photosystems (HL)
- ★ Light Dependent Reactions (HL)
- Photophosphorylation (HL)
- ★ Light Independent Reactions (HL)
- ★ Interdependence of Photosynthetic Reactions (HL)



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# The Process of Photosynthesis

# **Transformation of Light Energy During Photosynthesis**

- Simple, inorganic compounds are converted into complex organic ones by photosynthesis
   The energy required is provided by light
- Photosynthesis occurs in autotrophic organisms such as **plants**, **algae** and **cyanobacteria**
- Photosynthesis is a form of energy conversion, from light energy to chemical energy, stored in biomass
- Energy is stored within the bonds of these organic compounds and provides most of the chemical energy needed for life processes in ecosystems

## Examiner Tip

Remember, energy is never created or destroyed; it is only ever converted from one form to another!





# Conversion of Carbon Dioxide to Glucose

- During photosynthesis, carbon dioxide is converted to glucose using hydrogen released when a water molecule is split
  - Oxygen is released as a waste product
- The reactants of photosynthesis are carbon dioxide and water
- The products of photosynthesis are glucose and oxygen

#### Reactants and products of photosynthesis diagram



#### Photosynthesis as it takes place in a leaf

• We can represent this chemical reaction in a word equation

#### Photosynthesis word equation







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## 💽 Examiner Tip

The glucose and oxygen formed during photosynthesis are the reactants of aerobic cell respiration while carbon dioxide and water released during respiration are used as the reactants of photosynthesis

Respiration is the process by which energy is released from organic molecules in living cells

## **Release of Oxygen**

- Photosynthesis is carried out in **plants**, algae and cyanobacteria
- The oxygen that is released comes from the water splitting process which also provides hydrogen to allow the synthesis of glucose
- The paths of the oxygen and hydrogen can be seen more clearly when looking at the chemical symbol equation for photosynthesis

#### Chemical symbol equation for photosynthesis



# Examiner Tip

Note that you are only expected to know the word equation for photosynthesis for exam purposes



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# Separating Photosynthetic Pigments: Skills

## Separating Photosynthetic Pigments: Skills

#### Separation of photosynthetic pigments by chromatography

- Plants contain several different photosynthetic pigments, which absorb different wavelengths of light
- There are two groups of pigments: chlorophylls and carotenoids
- Carotenoids surround the chlorophyll and absorb both similar and different wavelengths of light to chlorophyll
  - This expands the range of wavelengths that can be absorbed from light for use in photosynthesis

Pigment group	Name of pigment	Colour of pigment
Chlorophylls	Chlorophyll a Chlorophyll b	Blue- green Yellow - green
Carotenoids	β carotene Xanthophyll	Orange Yellow

#### **Chloroplast Pigments Table**

- Chlorophylls absorb wavelengths in the blue-violet and red regions of the light spectrum
  - They reflect green light, causing plants to appear green
- Carotenoids absorb wavelengths of light mainly in the blue-violet region of the spectrum

#### Page 5 of 43



#### Page 6 of 43

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- Leaf sample
- Distilled water
- Pestle and mortar
- Filter paper
- Capillary tube
- Chromatography solvent
- Propanone
- Pencil
- Ruler

#### Method

- Draw a straight line in pencil approximately 1cm above the bottom of the filter paper being used
  - **Do not use a pen** as the ink will separate into pigments within the experiment and obscure the results
- Cut a section of leaf and place it in a mortar
  - It is important to choose a healthy leaf that has been in direct sunlight so you can be sure it contains many active photosynthetic cells
- Add 20 drops of propanone and use the pestle to grind up the leaf sample and release the pigments
  - Propanone is an organic solvent and therefore fats, such as the lipid membrane, dissolve in it
  - The combination of propanone and mechanical pressure breaks down the cell and chloroplasts to **release the pigments**
- Extract some of the pigment using a capillary tube and spot it onto the centre of the pencil line you have drawn
- Suspend the paper in the chromatography solvent so that the level of the solvent is below the pencil line and leave the paper until the solvent has reached the top of the paper
  - The mixture is **dissolved** in the **solvent** (called the mobile phase) and the dissolved mixture then passes through a static material (called the stationary phase)
- Remove the paper from the solvent and draw a pencil line marking where the solvent moved up to
  - The pigment should have separated out and there should be different spots on the paper at different heights above the pencil line, these are the separate pigments
- Calculate the R<sub>f</sub> value for each spot

# $R_{f}$ value = $\frac{\text{distance travelled by component (pigment)}}{\text{distance travelled by the solvent}}$

Always measure to the centre of each spot

## Results

#### Page 7 of 43



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- Chromatography can be used to separate and identify chloroplast pigments that have been extracted from a leaf as each pigment will have a unique R<sub>f</sub> value
- The R<sub>f</sub> value demonstrates how far a dissolved pigment travels through the stationary phase
  - Molecules with a higher affinity to the stationary phase, such as large molecules, will travel slower and therefore have a smaller R<sub>f</sub> value
  - Molecules that are more soluble in the mobile phase will travel faster and therefore have a larger R<sub>f</sub> value
- Although specific R<sub>f</sub> values depend on the solvent that is being used, in general:
  - Carotenoids have the highest R<sub>f</sub> values (usually close to 1)
  - Chlorophyll b has a much lower Rf value
  - Chlorophyll a has an R<sub>f</sub> value somewhere between those of carotenoids and chlorophyll b
  - Small R<sub>f</sub> values indicate the pigment is less soluble and/or larger in size





**Your notes** 



Paper chromatography is used to separate photosynthetic pigments. These pigments can be identified by their R<sub>f</sub> values. In this example, a line of the mixture (rather than a spot) is added to the paper.

#### Limitations

- Paper chromatography is not as specific as other chromatography techniques
  - It is sufficient to separate and distinguish different pigments and to calculate their R<sub>f</sub> value
- Chromatography does not give data on the amount of each pigment present or the wavelengths that they absorb
  - Colorimetry can be used to calculate these values

## 😧 Examiner Tip

Remember – the pigments themselves have colour (as described in the table). This is different from the colours of light that they *absorb*. You don't have to remember specific R<sub>f</sub> values, just know that they differ between each type of pigment.

# **Absorption Spectra**

# **Absorption Spectra**

- Light is made up of a mixture of all the **visible wavelengths** to include red, orange, yellow, green, blue, indigo and violet
- An **absorption spectrum** is a graph that shows the **absorbance** of different wavelengths of light by a particular pigment in the chlorophyll
- Within the chlorophyll, light energy results in the **excitation of electrons** which triggers transfer of electrons leading to a series of reactions which make up the process of **photosynthesis** 
  - During photosynthesis, light energy is transformed to chemical energy when glucose is formed
- Chlorophylls absorb wavelengths in the blue-violet and red regions of the light spectrum
- Carotenoids absorb wavelengths of light mainly in the blue-violet region of the spectrum
- The chemical structure of these molecules determines the wavelengths of light that can be absorbed
- The green part of the spectrum is largely **reflected** from the leaf and this is why leaves usually appear green









#### Absorption spectra of chlorophyll A, chlorophyll B and carotenoid pigments



# Absorption & Action Spectra: Skills

# **Comparing Absorption & Action Spectra**

### What is an action spectrum?

- An action spectrum is a graph that shows the rate of photosynthesis at different wavelengths of light
- The rate of photosynthesis is highest at the blue-violet and red regions of the light spectrum, as these are the wavelengths of light that plants can absorb (i.e. the wavelengths of light that chlorophylls and carotenoids can absorb)

#### Diagram to show the action spectrum of chlorophyll pigments



The photosynthetic action spectrum shows the rate of photosynthesis at different wavelengths of light

## Comparing action and absorption spectra

• There is a strong **correlation** between the cumulative absorption spectra of all pigments and the action spectrum:

#### Page 12 of 43



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**Your notes** 

- Both graphs have two main peaks at the blue-violet region and the red region of the light spectrum which supports the idea that the most light energy is absorbed at these wavelengths leading to the fastest rate of photosynthesis
- Both graphs have a trough in the green-yellow region of the light spectrum which supports the idea that the least light energy is absorbed at these wavelengths leading to the slowest rate of photosynthesis

PHOTOSYNTHETIC RATE / ABSORPTION RATE 100 80 60 40 20 0 700 400 500 600 WAVELENGTH (nm) KEY = ACTION SPECTRUM = CHLOROPHYLL A =  $\beta$ -CAROTENE = ABSORPTION SPECTRA = CHLOROPHYLL B Copyright © Save My Exams. All Rights Reserved

Diagram to show the correlation between action and absorption spectra



#### Determining the rate of photosynthesis

Page 13 of 43

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- The rate of photosynthesis can be determined by measuring the volume of oxygen produced or the carbon dioxide consumption at different wavelengths of light
- An experiment can be set up similar to the one investigating the effect of light intensity on photosynthesis
  - Remember that the lamp should be **kept the same distance** from the pondweed as we are investigating the effect of different wavelengths of light only
  - Place different colour filters (covering the full light spectrum) in front of the lamp to change the colour of light the pondweed is exposed to
  - Measure the volume of oxygen produced or the number of bubbles released from the pondweed per minute for each colour
    - Include an experiment with no filter in front of the lamp to investigate the effect of white light on the rate of photosynthesis
  - Repeat the experiment several times to obtain reliable results

### Drawing an action spectrum for photosynthesis

## Step 1: Draw and label the axes

- Draw an x-axis
- Label the axis **wavelength**
- Add the units / nm
- Make 400 the smallest value and 700 the largest value
  - Label 500 and 600 nm on the x-axis
- Draw a y-axis
- Label it Rate of photosynthesis / % of maximum rate
- Make 0 the lowest value and 100 the highest value
  - No units are required because the y-axis is showing a percentage scale

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# Limiting Factors of Photosynthesis: Skills

# **Limiting Factors**

- An **aquatic plant** such as *Elodea* or *Cabomba* is a good choice for investigating photosynthesis in plants, because the rate of photosynthesis can be measured by **counting oxygen bubbles** that come off a cutting of this plant
  - Oxygen output from terrestrial plants (that grow on land) would not be observable

### NOS: Hypotheses are provisional explanations that require repeated testing

- A hypothesis is a proposed explanation for an idea which may be true or false
- In an investigation the hypothesis can be tested through observations or experiments to provide either support or opposition to the proposed hypothesis
- The following investigation looks at the effect of limiting factors on the rate of photosynthesis
- A suggested **hypothesis** for an investigation into the effect of light intensity on photosynthesis could be:
  - Light intensity will have an effect on the rate of photosynthesis

### Identifying the variables in an investigation

- When designing an experiment it is crucial that all variables (apart from the independent and dependent variables being investigated) are controlled
  - The independent variable is the factor that is **deliberately manipulated** between a specific range throughout the experiment
  - The dependent variable is the factor that is **measured** during the experiment (to see if it is affected by the changes to the independent variable)
  - Other variables must be **controlled** so that it can be said the independent variable is the only factor affecting the dependent variable during the experiment
- Changes in light intensity, carbon dioxide concentration and temperature are all limiting factors that affect the rate of photosynthesis and can be altered experimentally to measure the effect on the rate of photosynthesis
  - Any of these limiting factors could be selected as the independent variable in the investigation

## Effect of light intensity - experimental design

- Basic Experimental Setup
  - Aquatic plant cutting in water
  - Powdered sodium hydrogencarbonate (NaHCO<sub>3</sub>)
  - Glass funnel
  - Boiling tube
  - Lamp for illumination
  - Blass tank filled with water



**Your notes** 



## Method

- Place a piece of aquatic plant (Elodea or Cabomba are often used), into a beaker of water
- Place a lamp a set distance from the plant
- Record the number of bubbles observed in three minutes
- Repeat these steps for different distances between the lamp and plant

#### Improvements

- Use a **gas syringe** to collect and measure the volume of gas produced
- For **reliability** of data, **repeat** the experiment at least twice for each distance and calculate the mean number of bubbles
- Use of a **data logger** to measure results continuously

### Variables to Be Controlled

#### Page 18 of 43

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- Temperature
  - The glass tank filled with water absorbs any heat that is emitted from the lamp
  - Modern LED bulbs can be used as they give off less heat than filament bulbs
- CO<sub>2</sub> concentration
  - The water used around the plant is first **boiled and re-cooled** to remove any dissolved carbon dioxide
  - A set mass of sodium hydrogencarbonate is added to the water that surrounds the plant to make the concentration approx. 0.1 mol dm<sup>-3</sup>
    - This will ensure that the carbon dioxide concentration is not limiting the rate of photosynthesis

#### Results

- A graph of the **number of bubbles produced per minute** against the **distance between the lamp and the plant** used can be drawn to see the pattern or trend
  - Distance between the lamp and the plant is linked to the light intensity



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- A graph can also be drawn showing the effect of different light intensities on the rate of photosynthesis
- It can be seen that:

- As light intensity increases so too does the rate of photosynthesis (positive correlation)
  - At this stage light intensity is the limiting factor
- At some point, there will be no further increase in the rate of photosynthesis if the light intensity is increased
  - Now temperature or carbon dioxide concentration may be limiting factors



 A graph of the effect of carbon dioxide concentration against the rate of photosynthesis shows a similar trend to what was observed with light intensity

#### Page 20 of 43



**Your notes** 



#### Page 21 of 43



# **Carbon Dioxide Enrichment Experiments**

# **Carbon Dioxide Enrichment Experiments**

- Future rates of photosynthesis and plant growth can be predicted using experiments such as
  - enclosed greenhouse experiments
  - free air carbon dioxide enrichment experiments (FACE)
- Due to the impact of global warming already documented and rising levels of greenhouse gases, including carbon dioxide, it is fundamental that studies are carried out to establish the effect of carbon dioxide on plant growth and photosynthesis to develop a clearer idea of the potential future risks that we may encounter

### **Enclosed greenhouse experiments**

- Monitoring photosynthesis and growth can be done using an enclosed greenhouse or polytunnel set up
- This allows variables to be **manipulated** or **controlled** in order to establish the impact of different factors
- Only small species that can be contained in a greenhouse can be studied using this method
- Variables that would be manipulated might include
  - light
  - carbon dioxide
  - temperature
  - wavelengths of light
- Other variables should be controlled so as to ensure that the effect of only **one variable** is being considered at any one time

## Free air carbon dioxide enrichment experiments (FACE)

- These experiments are carried out in natural ecosystems where carbon dioxide is pumped into the area to increase the localised carbon dioxide concentrations
- This set up allows larger plants and trees to be studied
- Other variables cannot be controlled in these scenarios but they can be monitored to establish any relationships that may become apparent in the data

# NOS: Finding methods for careful control of variables is part of experimental design

- In an experiment, a variable is any factor that could change or be changed
  - The independent variable: the only variable that should be changed throughout an experiment
  - The **controlled/confounding variables**: any other variables that may affect the results of the experiment that need to be controlled or monitored
  - The **dependent variable**: the variable that is measured to determine the outcome of an experiment (the results)
- It is essential that any variable that may affect the outcome of an experiment is controlled in order for the results to be valid

#### Page 23 of 43





- **Preliminary research** and preliminary studies can be used to **identify variables** within an experiment and to determine ways of controlling these variables effectively
- The science surrounding the issue/problem being investigated is likely to contain information about different factors or variables that may exist



# Photosystems (HL)

## Photosystems

### What are photosystems?

- Chloroplasts contains the pigment chlorophyll, plus other accessory pigments
- These are grouped together as structures called photosystems which are located in the thylakoid membranes in cyanobacteria and photosynthetic eukaryotes
- Photosystems contain many chlorophyll molecules and accessory pigments (carotene and xanthophylls) as well as 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 and accessory pigments within Photosystem II absorb light energy, in the form of photons, and pass it to a chlorophyll molecule in **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
   Diagram to show excitation of electrons in a photosystem



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

#### Page 25 of 43



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## 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.

# Advantages of Photosystems

### Why are there multiple pigments in a photosystem?

- In each photosystem, the presence of many different types of pigment, each with a specific role, allows the photosystem to efficiently absorb light of different wavelengths
  - The structured arrangement of these pigments and accessory pigments allows for electrons to be excited in a controlled manner
  - These can then be directed along the electron transport chain
- All the pigments in photosystem I and II are required in order for photosynthesis to occur
  - A single pigment molecule would not be able to perform any part of photosynthesis

# Table to show the pigments involved in light harvesting in the light dependent stage of photosynthesis

Pigment	Role
Chlorophyll (a, b)	Absorb wavelengths of light in the blue to violet and red regions of the spectrum
Carotenoid accessory pigments (xanthophyll, carotene)	Absorb wavelengths of light in the blue to violet region of the spectrum
Light harvesting complex proteins: enzymes	To catalyse: <ul> <li>formation of ATP from ADP + P<sub>i</sub></li> <li>reduction of NADP<sup>+</sup> to NADPH + H<sup>+</sup></li> </ul>
Light harvesting complex proteins: electron carrier molecules	Pass electrons down an electron transport chain



#### Page 26 of 43

# Light Dependent Reactions (HL)

# 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

### Where do the light dependent reactions take place?

- Both stages of photosynthesis 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

#### What happens in the light-dependent reaction?

Three key processes which occur during the light-dependent reaction in the thylakoid membrane include

- **Photolysis**: The splitting of a water molecule using light energy
  - This occurs in photosystem II
- **Chemiosmosis**: The synthesis of ATP using an electrochemical gradient produced by H<sup>+</sup> protons
  - The proton gradient forms across the thylakoid membrane when protons are pumped from the chloroplast matrix into the thylakoid spaces
- Reduction of NADP: NADP<sup>+</sup> accepts electrons (from photophosphorylation) and H+ protons to become NADPH
  - This occurs in photosystem I

#### Products of the light-dependent reaction

- During the light-dependent reaction light energy is converted into chemical energy in the form of **ATP** and **reduced NADP**
- Oxygen is given off as a waste product of the light-dependent reaction
- The useful products of the light-dependent reaction are transferred to the light-independent reaction within the chloroplast

# 😧 Examiner Tip

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

# Diagram to show the location of the light dependent and light independent stages of photosynthesis

#### Page 27 of 43



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Page 28 of 43

# Photolysis

### Photolysis and the light-dependent reaction

- Photolysis occurs in Photosystem II during the light-dependent reaction of photosynthesis
  - 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 is released as a waste product, it 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<sub>2</sub>O → O<sub>2</sub> + 4H<sup>+</sup> + 4e<sup>-</sup>
- 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

#### The effect of oxygen

- Changes to the Earth's atmosphere, oceans and rock deposition occur due to photosynthesis, and more specifically **photolysis**
- The first life forms emerged around 4 billion years ago
  - At the time, there was **no oxygen in the atmosphere**
- About 3.5 billion years ago photosynthetic prokaryotes became the first organisms to carry out photosynthesis
  - This began the release of oxygen into the atmosphere
- Millions of years later algae and plants evolved and also carried out photosynthesis
- Around 2.2 billion years ago, the oxygen concentration in the atmosphere reached 2%
  - This is known as the Great Oxidation Event
- Other changes to the Earth occurred due to photosynthesis
  - Minerals in the oceans were oxidised
  - Photosynthetic bacteria released oxygen into the ocean
  - When dissolved iron was oxidised it formed iron oxide which is a red precipitate that lies on the sea bed
  - Over time a distinctive rock formation was produced the banded iron formation. Layers of red iron oxide alternate with other mineral oxides
  - Banded iron formations are the most important source of iron ores (and consequently our supply of steel)
  - Methane and CO<sub>2</sub> levels in the air fell, which resulted in an **Ice Age** 
    - This is because methane and CO<sub>2</sub> are important greenhouse gases
- By 600 million years ago, life had evolved into large multicellular organisms, many of which were photosynthetic (plants)
  - This pushed the oxygen concentration of the air up to 20%, **peaking at 35%** 300 million years ago
  - This contributed to the large size of the animals that roamed the Earth at that time
- The current atmospheric oxygen level is around 21%, due to **increased human activity**, e.g. burning of fossil fuels, deforestation which remove oxygen from the atmosphere

#### Page 29 of 43



# Photophosphorylation (HL)

# Chemiosmosis in Photosynthesis

## Types of photophosphorylation

- The photophosphorylation of ADP to ATP can be cyclic or non-cyclic, depending on the pattern of electron flow in photosystem I or photosystem II or both
  - In cyclic photophosphorylation, only photosystem l is involved
  - In non-cyclic photophosphorylation, both photosystem I and photosystem II are involved

## Cyclic photophosphorylation

- Cyclic photophosphorylation involves photosystem I (PSI) only
- Light is absorbed by photosystem I (located in the thylakoid membrane) and passed to the photosystem I primary pigment (P700)
- An electron in the primary pigment molecule (i.e. the chlorophyll molecule) is excited to a higher energy level and is emitted from the chlorophyll molecule in a process known as photoactivation
- This excited electron is captured by an electron acceptor, transported via a **chain of electron carriers** known as an **electron transport chain** before being passed back to the chlorophyll molecule in photosystem I (hence: cyclic)
- As electrons pass through the electron transport chain they provide energy to transport protons (H<sup>+</sup>) from the stroma to the thylakoid lumen via a **proton pump**
- A build-up of protons in the thylakoid lumen can then be used to drive the synthesis of ATP from ADP and an inorganic phosphate group (P<sub>i</sub>) by the process of chemiosmosis
- **Chemiosmosis** is the movement of chemicals (protons) down their concentration gradient, the energy released from this can be used by ATP synthase to synthesise ATP
- The ATP then passes to the light-independent reactions

## Cyclic photophosphorylation diagram





- The electron transport chain occurs on the thylakoid membranes within the chloroplast
- Thylakoid membranes contain the following structures:
  - Photosystem II
  - ATP synthase
  - A series of electron carriers

#### Page 31 of 43

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- Photosystem I
- An electron acceptor carries a pair of excited electrons from Photosystem II to the start of a chain of electron carriers
- The electron carriers undergo a series of redox reactions as electrons are gained and lost from each carrier
- Excited electrons gradually release their energy as they pass through the electron carriers which is used to generate a **proton gradient**
- The excitation of the electrons falls and they are eventually picked up by the reaction centre in Photosystem I
- Finally the pair of electrons are used to **reduce NADP** (along with protons from the photolysis of water) which is then passed into the light-independent reaction
- The pathway of electrons is linear, photophosphorylation is referred to as non-cyclic photophosphorylation
- ATP and reduced NADP are the main products of photophosphorylation and are immediately passed to the light-independent reaction

Non-cyclic photophosphorylation diagram





Page 33 of 43

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





3

4 EXCITED ELECTRONS FROM PHOTOSYSTEM II ARE PASSED TO PHOTOSYSTEM I VIA AN ELECTRON TRANSPORT CHAIN, RELEASING SUFFICIENT ENERGY TO SYNTHESISE ATP



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

6 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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Non cyclic phosphorylation involving the electron transport chain and the production of ATP and reduced NADP

# 😧 Examiner Tip

Make sure you know the difference between the two forms of photophosphorylation! Cyclic photophosphorylation differs from non-cyclic photophosphorylation in two key ways:

- Cyclic photophosphorylation **only** involves photosystem I (whereas non-cyclic photophosphorylation involves photosystems I and II)
- Cyclic photophosphorylation does **not** produce reduced NADP (whereas non-cyclic photophosphorylation does)

### Chemiosmosis

 During the light dependent stages of photosynthesis, ATP is synthesizes from ADP + P<sub>i</sub> using energy released from the movement of H<sup>+</sup> protons down an electrochemical gradient

### Forming a 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 intermembrane space (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 contributes to the proton gradient

#### Synthesis of ATP

- 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

### Chemiosmosis diagram

#### Page 35 of 43





Remember – the oxygen produced during the photolysis of water is a waste product of this process. The hydrogen ions and electrons produced during the photolysis of water are useful products. The electrons replace those that have been lost from the primary pigment molecule of photosystem II (as photosystem II passes its electrons on to photosystem I). The hydrogen ions combine with the electrons from photosystem I to form reduced NADP (NADPH).

**Your notes** 

# **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<sup>+</sup> to NADPH
    - NADP<sup>+</sup> + 2H<sup>+</sup> + 2e<sup>-</sup>  $\rightarrow$  NADPH + H<sup>+</sup>
  - Reduced NADP now carries a pair of electrons and can be passed into the light-independent reactions of photosynthesis

#### Diagram to show the reduction of NADP in the light dependent stage of photosynthesis



# Light Independent Reactions (HL)

# **Carbon Fixation**

### 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

### 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
  - This is the most abundant enzyme on Earth
  - It works relatively slowly, therefore high concentrations of it is needed in the stroma
  - It is not effective in low carbon dioxide concentrations
- Glycerate-3-phosphate is then used in the next step of the cycle

### Page 38 of 43



# Synthesis of Triose Phosphate

- 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 as 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**

- **One sixth** of the triose phosphate that is generated will be used in the synthesis of organic compounds, such as glucose
- The remaining five sixths of triose phosphate are used to regenerate the five-carbon compound ribulose bisphosphate (RuBP)
- Five molecules of triose phosphate are converted to three molecules of 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

#### Light-independent stage of photosynthesis diagram





Page 41 of 43



# Synthesis of Carbon Compounds

- Carbon containing compounds, to include carbohydrates, proteins and lipids, all rely on the **products** of the Calvin cycle in combination with other mineral nutrients
- Whilst glucose is the key respiratory substrate which sustains the metabolism of green plants, other required substances can be produced from the further metabolism of TP, for example:
  - Triose phosphate can be sent through the **glycolysis and link reaction** pathways to produce acetyl coenzyme A which can then be conjoined to make **fatty acids**
  - Triose phosphate can be used to make **glycerol**
  - Glycerol can then be joined to fatty acids to make triglycerides
  - All 20 amino acids can be produced in plants using nitrate or ammonium ions



# Interdependence of Photosynthetic Reactions (HL)

# Interdependence of Photosynthetic Reactions

- The light dependent reaction and light independent reaction are **interdependent**
- This means that one cannot occur without the other
  - Products from the light dependent reaction (reduced NADP and ATP) are directly used in the Calvin cycle to produce carbohydrates
    - This means that in low light intensity, the products are produced at a slower rate which limits the conversion of GP to TP
  - Once the reduced NADP has been oxidised in the Calvin cycle, NADP is returned to the light dependent stage to accept electrons at the end of the electron transport chain
    - In high light intensity, the light dependent reactions occur more quickly, providing more reduced NADP and ATP to drive the Calvin cycle
    - However, if NADP is not returned to the light dependent stage quickly enough, then the process will be restricted
- Carbon dioxide, in the form of hydrogen carbonate ions (HCO<sub>3</sub>-), accepts protons from photosystem II when water is split during photolysis
  - Hydrogen carbonate also plays an important role in the functioning of the electron transport chain
- A lack of carbon dioxide (and thus hydrogen carbonate) will not only prevent carbon fixation from occurring but also **prevent photosystem II** from functioning

