Photosynthesis and Respiration

Photosynthesis: The Light Reactions

Chapter 7
pp. 163-197
5th Ed. Taiz and Zeiger 2010

Objectives

• Role of light in photosynthesis
• Structure of the photosynthetic apparatus
• Excitation of chlorophyll by light
• Synthesis of ATP and NADPH
Efficiency of energy conversion (%)

- Ordinary agricultural practices: 0.1 – 1.0%
- Intensive agricultural conditions: 2.0 – 2.5%
- Brief periods (some crop plants): 6.0 – 10.0%
- Laboratory conditions: 20 – 25%
- Theoretical maximum efficiency of energy conversion: 25 – 30%

Major reasons for low energy conversions:
- lack of water
- low or high temperatures
- deficiencies of inorganic nutrients
- pests (weeds, fungi, bacteria, rodents)
- faulty seed
- poor cultural practices

What Is Photosynthesis?

- Term literally means “synthesis using sun”.
- The use of solar energy to synthesize carbon compounds that cannot be formed without input of energy.
Photosynthesis

• Light energy drives the synthesis of carbohydrates from carbon dioxide and water with the generation of oxygen:

\[ 6 \text{CO}_2 + 6 \text{H}_2\text{O} \xrightarrow{\text{Light}} \text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2 \]

• Solar energy used to oxidize water; thereby releases oxygen and reduces CO\(_2\); forms large carbon compounds, primarily sugars.

Photosynthesis

• Mesophyll of leaves is the most active photosynthetic tissue in higher plants

• Mesophyll cells contain many chloroplasts

• Chloroplasts possess specialized light-absorbing green pigments, chlorophylls

Photosynthesis

• Complex series of reactions that culminate in reduction of CO\(_2\)

• These reactions include:
  -- Thylakoid (light) reactions
  -- Carbon fixation (dark) reactions
Photosynthesis

• Light reactions take place in the specialized internal membranes of the chloroplasts called thylakoids.

• End products of these thylakoid reactions are the high energy compounds ATP and NADPH.

Photosynthesis

• ATP and NADPH are used for synthesis of sugars in the carbon fixation reactions.

• These synthetic processes take place in the stroma of the chloroplasts, the aqueous region that surrounds the thylakoid.

Photosynthesis

• Light energy is converted into chemical energy by two different functional units called photosystems (PS I and PS II).

• Absorbed light energy is used to power the transfer of electrons through a series of compounds that act as electron donors and electron acceptors.
Photosynthesis

- What ultimately happens to the majority of the electrons?
  - Used to reduce NADP+ to NADPH and oxidize H₂O to O₂.
  - Light energy is also used to generate a proton motive force (PMF) across the thylakoid membrane, which is used to synthesize ATP.

Nature of Light

- Light has characteristics of both a particle and a wave.
  - Wave → characterized by a wavelength (λ)
    - λ = lambda
    - A wavelength is the distance between successive wave crests
  - Frequency (ν) → number of wave crests that pass an observer in a given time.
    - ν = frequency = nu

Nature of Light

Relationship of Wavelength, Frequency, and Speed of Any Wave

- Expressed by:
  - c = λν
  - c = speed of wave = speed of light in this case, 3.0 x 10⁸ ms⁻¹
  - λ = wavelength
  - ν = frequency
Nature of Light
• Light is also a particle, which we call a photon.
• Photon contains an amount of energy termed quantum.
• Energy content of light is not continuous but rather is delivered in these discrete packets, the quanta.

Nature of Light
• Energy ($E$) of a photon depends on frequency of the light.
  – Expressed by Planck’s Law:
    • $E = h\nu$
    • $E = $ Energy
    • $h =$ Planck’s constant ($6.626 \times 10^{-34} \text{ J s}$)
    • $\nu$ (nu) = frequency

Electromagnetic Spectrum
• Sunlight is like a rain of photons of different frequencies.
• Our eyes are sensitive to only a small range of frequencies, the visible region.
Electromagnetic Spectrum

- Visible region extends from about 400 nm (violet) to 700 nm (red)

Wavelength (λ) and frequency (ν) are inversely related.

- Short wavelength light (high frequency) has a high energy content; UV portion of spectrum.
- Long wavelength light (low frequency) has a low energy content; infra-red region of spectrum.

Absorption Spectrum

- Displays the amount of light energy taken up or absorbed by a molecule or substance as a function of the wavelength of the light.
Absorption Spectrum

• Curve A - energy output of sun as a function of a wavelength.

• Curve B - energy that strikes surface of Earth.

• Sharp valleys in the infra-red region beyond 700 nm represent absorption of solar energy by molecules in the atmosphere → chiefly water vapor.

Absorption Spectrum

• Curve C - absorption spectrum of chlorophyll
  – Absorbs strongly in blue (~430 nm) and red (~660nm) portions

• Green is not efficiently absorbed; thus, reflected into our eyes. (chlorophyll appears green to our eyes because it absorbs mainly in the red and blue parts of the spectrum)

Minolta SPAD 502 Meter
Electronic State of Molecules
Changed When Light is Absorbed or Emitted

• Absorption of light represented by:
  – Chl + \( h\nu \rightarrow \text{Chl}^* \)
  – Chl \rightarrow \text{chlorophyll in its lowest-energy, or ground state}
  – \( h\nu = \text{photon} \)
  – \( \text{Chl}^* = \text{chlorophyll that has transitioned to a higher-energy, or excited state} \)

Electronic State of Molecules
Changed When Light is Absorbed or Emitted

• Distribution of electrons in the excited molecule somewhat different from distribution in the ground-state molecule.

Electronic State of Molecules
Changed When Light is Absorbed or Emitted

• Absorption of blue light excites chlorophyll to a higher energy state than does red.
  • Why? Because energy of photons is higher when the wavelength is shorter.
**Light Absorption and Emission by Chlorophyll**

- Absorption or emission of light indicated by vertical lines that connect the ground state with excited electron states.

- Blue and red absorption bands of chlorophyll (which absorb blue and red photons, respectively) correspond to the upward vertical arrows → signifies that energy absorbed from light causes the molecules to change from the ground state to the excited state.

**Fluorescence** is indicated by the downward-pointing arrow in which a molecule goes from the lowest excited state to the ground state while re-emitting energy as a photon.

**The long-wavelength (red) absorption band of chlorophyll corresponds to light that has energy required to cause transition from the ground state to the first excited state.**
**Light Absorption and Emission by Chlorophyll**

- The short-wavelength (blue) absorption band corresponds to a transition to a higher excited state.

**Electronic State of Molecules Changed When Light is Absorbed or Emitted**

- Chlorophyll is extremely unstable in the higher excited state.
- In this state, chlorophyll very rapidly gives up some of its energy to the surroundings as heat.
- Enters the lowest excited state.
- Stable for a maximum of several nanoseconds (10^-9 s).
- Therefore any process capturing the energy from chlorophyll must be extremely rapid.

**Pathways for Disposing of Energy From Chlorophyll**

- In the lowest excited state, excited chlorophyll has four alternative pathways to dispose of its available energy.

1. Re-emit a photon and return to its ground state → a process known as fluorescence.
Fluorescence

The wavelength of fluorescence is slightly longer (and of lower energy) than the wavelength of absorption.

Why?

A portion of the excitation energy is converted into heat before the fluorescent photon is emitted.

Chlorophylls fluoresce in the red region of the spectrum.

Pathways for Disposing of Energy From Chlorophyll

- Alternative pathways:

  2. Excited chlorophyll can return to its ground state by directly converting excitation energy into heat

    - No emission of a photon

  3. Chlorophyll may participate in energy transfer.

    - In this process, excited chlorophyll transfers its energy to another molecule.

  4. Photochemistry — energy of the excited state causes chemical reactions to occur

    - Among fastest known chemical reactions.
Photosynthetic Pigments

• Absorb light that powers photosynthesis

• All pigments active in photosynthesis are found in chloroplast.

• Chlorophylls $a$ and $b$ are abundant in green plants.

Photosynthetic Pigments

• All chlorophylls have a complex ring structure that is chemically related to porphyrin – like groups in hemoglobin and cytochromes.

• A long hydrocarbon tail is almost always attached to the ring structure.

Photosynthetic Pigments

• A magnesium atom (Mg) is found in the center of the porphyrin-like ring structure.

• The hydrocarbon tail anchors the chlorophyll molecules in the photosynthetic membrane.
Photosynthetic Pigments

• The porphyrin-like ring is the site of the electron rearrangements that occur when chlorophyll is excited.
  – also the site of the unpaired electrons when it is oxidized or reduced.

Photosynthetic Pigments

• Carotenoids in photosynthetic organisms are all linear molecules with multiple conjugated double bonds

• Absorption bands in the 400 to 500 nm region give carotenoids their characteristic orange color
  – e.g. carrots, the carotenoid β-carotene

Photosynthetic Pigments

• Carotenoids are integral constituents of the thylakoid membrane intimately associated with both antennas and reaction center pigment proteins.

• Because light absorbed by carotenoids is transferred to chlorophyll for photosynthesis they are called accessory pigments.
Carotenoids Protect Against Photo-oxidation

• Photo-oxidation occurs when excited chlorophyll transforms oxygen into high-energy radicals.

• High-energy radicals attract hydrogen from nearby molecules, thereby destroying the molecules and killing the cells.

• Herbicides kill plants by blocking synthesis of carotenoids e.g. triazoles (Command, Zorial).

Carotenoids Protect Against Photo-oxidation

• Plants on left treated with herbicide that blocks carotenoid synthesis.

• Consequently plants became photo-oxidized (bleached) and died when grown in light.

• Untreated plant on right made carotenoids and therefore were not photo-oxidized by light.

Action Spectra

• Relate light absorption to photosynthetic activity.

• Depicts the magnitude of a biological system to light, as a function of wavelength.

• For example, an action spectrum for Ps can be constructed from measurement of O₂ evolution at different wavelengths.
Comparison of Action Spectrum with Absorption Spectrum

• Action spectrum is measured by plotting the response of light to oxygen evolution, as a function of wavelength.

Comparison of Action Spectrum with Absorption Spectrum

• Absorption spectrum and action spectrum match if the pigment used to obtain the absorption spectrum is the same as those that cause the response.
  e.g. Intact chloroplasts

Schematic Diagram of Action Spectrum

• T.W. Engelmann → projected light spectrum onto spiral chloroplasts of filamentous green algaSpirogyra
Observed that oxygen-seeking bacteria introduced into the system collected in region of spectrum, where chlorophyll pigments absorb.

This action spectrum was the first indication of effectiveness of light absorbed by accessory pigments in driving photosynthesis.

Majority of pigments serve as an antenna complex
- Collect light and transfer energy to the reaction center complex
- The chemical oxidation and reduction reactions leading to long-term energy storage take place here.
Complexes Containing Light-Harvesting Antennas and Photochemical Reaction Centers

- This illustrates basic concept of energy transfer.
- Many pigments serve together as an antenna to collect light and transfer its energy to the reaction center.

Complexes Containing Light-Harvesting Antennas and Photochemical Reaction Centers

- In the reaction center chemical reactions store some of the energy by transferring electrons from a chlorophyll pigment to an electron acceptor molecule.

Complexes Containing Light-Harvesting Antennas and Photochemical Reaction Centers

- Chlorophyll is reduced again by an electron donor.
- Transfer of energy in the antenna is a purely physical phenomenon.
- Involves no chemical changes.
Benefit from Division of Labor

• Is there a benefit from the division of labor between antenna and reaction center pigments?
  – Under bright sunlight a chlorophyll molecule absorbs only a few photons each second.
  – If every chlorophyll had a complete reaction center associated with it, the enzymes making up this system would be idle most of the time.
  – Thus, many pigments sending energy into a common reaction center keeps system active a large fraction of the time.

Emerson and Arnold, 1932

• Provides first evidence for cooperation of many chlorophyll molecules in energy conversion.
• Delivered very brief flashes of light to suspension of green alga *Chlorella pyrenoidosa*
• Measure O₂ production

Emerson and Arnold, 1932

• Varied the energy of the light flashes and found that at high energies the O₂ production did not increase as more intense flashes were given.
• Photosynthetic system was saturated with light.
• Under saturating conditions, only one molecule of O₂ was produced for each 2500 chlorophyll molecules in the sample.

We now know that several hundred pigments are associated with each reaction center and that each reaction center must operate four times to produce one molecule of oxygen.
Quantum Yield

• Information about fate of the excited state of the chlorophyll molecule
  – Process with highest rate is the one that most likely deactivates chlorophyll
  – Concept expressed quantitatively by way of quantum yield

Quantum yield (Φ) = \frac{Yield of photochemical products}{total number of quanta absorbed}

Quantum Yield

• The quantum yield (Φ) of a process in which molecules give up their excitation energy (or “decay”) is the fraction of excited molecules that decay in that pathway.

• Quantum yield can range from 0 to 1. At 0, process does not respond to light. At 1.0, every photon absorbed contributes to the process.

Quantum Yield

• “Phi” (Φ) of functional chloroplasts kept in dim lights is ~ 0.95
  • Φ of fluorescence is 0.05 or lower
  • Φ of other processes are negligible
  • Therefore vast majority of excited chlorophyll molecules lead to photochemistry.
The Chemical Reaction of Photosynthesis Is Driven by Light

\[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow (\text{CH}_2\text{O}) + \text{O}_2 \]

Light, plant

\( \text{CH}_2\text{O} \) = one-sixth of a glucose molecule.

- The photochemical quantum yield under optimum conditions is nearly 100%.
- The efficiency of the conversion of light into chemical energy is much less.
- Equilibrium for this reaction lies very far in the direction of the reactants \( \rightarrow \) means no spontaneous generations of glucose from \( \text{H}_2\text{O} \) and \( \text{CO}_2 \) without external energy being provided.

Example

- Red light of wavelength 680 nm is absorbed.
- The total energy input is 1760 kJ per mole of oxygen formed.
- This amount of energy is more than enough to drive the photosynthesis reaction, which has a standard free-energy change of +467 kJ mol\(^{-1}\).
- The efficiency of conversion of light energy at the optimal wavelength into chemical energy is therefore about 27%.
  
  \[ \frac{467}{1760} = 26.5\% \]

Quantum Efficiency versus Energy Efficiency

- No conflict between the fact that:
  - Photochemical quantum efficiency = 100%
  - Energy conversion efficiency = 27%
- Quantum efficiency is a measure of the fraction of absorbed photons that engage in photochemistry.
- Energy efficiency is a measure of how much energy in the absorbed photons is stored as chemical products.
Light Drives the Reduction of NADP and the Formation of ATP

- The overall process of photosynthesis is a redox chemical reaction.
  - Electrons are removed from one chemical species, thereby oxidizing it, and added to another species, thereby reducing it.
- Robert Hill found that in the light, chloroplast thylakoids reduce a variety of compounds, such as iron salts.
  \[4 \text{Fe}^{3+} + 2 \text{H}_2\text{O} \rightarrow 4 \text{Fe}^{2+} + \text{O}_2 + 4 \text{H}^+\]
- Iron salts serve as oxidants in place of CO₂

The Hill Reaction

- Hill Reaction: Illuminated chloroplasts evolve O₂ and reduce electron acceptors.
  \[2\text{H}_2\text{O} + 4 \text{Fe}^{3+} \underset{\text{illuminated}}{\rightarrow} \text{O}_2 + 4 \text{H}^+ + 4 \text{Fe}^{2+}\]
- Landmark in elucidation of photosynthesis for several reasons:
  - Dissected photosynthesis → showed that O₂ evolution can occur without reduction of CO₂.

The Hill Reaction (cont.)

- Confirmed evolved O₂ comes from H₂O rather than CO₂ because no CO₂ was present.
- Showed that isolated chloroplasts can perform a significant partial reaction of photosynthesis.
- Revealed that a primary event in photosynthesis was the light-activated transfer of an electron from one substance to another against a chemical potential.
Additional Confirmation that O$_2$ Evolved in Photosynthesis Comes from Water

- Use of heavy isotope of oxygen, namely $^{18}$O$_2$.

$$H_2^{18}O + CO_2 \xrightarrow{\text{light}} (CH_2O) + ^{18}O_2$$

Two Light Reactions Interact in Photosynthesis

- Investigations of the dependence of the rate of photosynthesis on the wavelength of incident light led to the discovery that chloroplasts contain two different photosystems.
- The photosynthetic rate (i.e., the rate of O$_2$ evolution) divided by the number of quanta absorbed gives the relative quantum efficiency of the process.
- For a single kind of photoreceptor, the quantum efficiency is expected to be independent of wavelength over its entire absorption band, although this is not the case in photosynthesis.

“Red Drop”

- In photosynthesis, the quantum efficiency drops sharply at wavelengths longer than 680 nm, although chlorophyll still absorbs light in the range from 680 to 700 nm.
Emerson Enhancement Effect

- The rate of photosynthesis when red and far-red light are given together is greater than the sum of the rates when given separately.
- Supports the concept that photosynthesis is carried out by two photochemical systems working in tandem but with slightly different wavelength optima.

Photosystem I and II Have Complementary Roles

- Photosystem I (PSI) preferentially absorbs far-red light of wavelengths > 680 nm (P700).
- Photosystem II (PSII) preferentially absorbs red light of 680 nm and is driven very poorly by far-red light (P680).
- PSI produces a strong reductant, capable of reducing NADP⁺, and a weak oxidant.
- PSII produces a very strong oxidant, capable of oxidizing water, and a weaker reductant than the one produced by PSI.

Photosystem I and II Have Complementary Roles

Interaction of photosystems I and II in photosynthesis by green plants.
Photosystem I and II Have Complementary Roles

- Z Scheme of photosynthesis. Z is for zigzag scheme; has become the basis for understanding O₂-evolving (oxygenic) photosynthetic organisms.

- Red-light absorbed by PSII produces a strong oxidant and a weak reductant.

- Far-red light absorbed by PSI produces a weak oxidant and a strong reductant.

- The strong oxidant generated by PSII oxidizes water, while the strong reductant generated by PSI reduces NADP⁺.

- P680 and P700 refer to the wavelength of maximum absorption of the reaction center chlorophylls in PSII and PSI, respectively.
Organization of the Photosynthetic Apparatus

- Chloroplasts of higher plants are surrounded by the inner and outer membranes (envelope).
- The region of the chloroplast that is inside the inner membrane and surrounds the thylakoid membranes is known as the stroma (site of carbon reactions).

Organization of the Photosynthetic Apparatus

- The thylakoid membranes are highly folded and appear in many pictures to be stacked like coins; in reality they form one or a few large interconnected membrane systems.
- The thylakoid membranes have a well-defined interior and exterior with respect to the stroma.
- The inner space within a thylakoid is known as the lumen.

The Chloroplast: The Site of Photosynthesis

- Thylakoids → the extensive system of internal membranes which present the most striking aspect of the chloroplast.
  - All chlorophyll is contained in this membrane system.
  - Site of light reactions of photosynthesis
- Stroma → the region of the chloroplast outside the thylakoids
  - Site of the carbon reduction reactions.
The Chloroplast: The Site of Photosynthesis

• Most of the thylakoids appear to be very closely associated with each other.
  – The stacked membranes are known as grana lamellae.
  – Each stack is known as a granum.
  – The exposed thylakoid membranes in which stacking is absent are known as stroma lamellae.

Organization of the Protein Complexes of the Thylakoid Membrane

• Photosystems I and II are spatially separated in the thylakoid membrane.
  – PSII reaction center, along with its antenna chlorophylls and associated electron transport proteins, is located predominately in the stacked regions of the thylakoid membrane (grana lamellae).

• PSI reaction center and its associated antenna pigments and electron transfer proteins, as well as the coupling factor enzyme that catalyzes the formation of ATP are found exclusively in the stroma lamellae and at the edges of the grana lamellae.

• Cytochrome b6f, the complex of the electron transport chain that connects the two photosystems, is evenly distributed between stroma and grana.
The Antenna Funnels Energy to the Reaction Center

- Antenna systems in higher plants generally consists of 200 to 300 chlorophyll per reaction center.
- Energy transfer in antenna complexes is very efficient: 95 to 99% of the photons absorbed by antenna pigments is transferred to the reaction center.

- The excited-state energy of pigments increases with distance from the reaction center.
- Pigments closer to the reaction center are lower in energy than those farther from the reaction center.

- This energy gradient ensures that excitation transfer toward the reaction center is energetically favorable.
- Excitation transfer back out to the peripheral portions of the antenna is energetically unfavorable.
The Antenna Funnels Energy to the Reaction Center

• Some energy is lost as heat to the environment by this process.

• However, under optimal conditions almost all the excitations absorbed in the antenna complexes can be delivered to the reaction center.

Mechanisms of Electron Transport

• In the Z-scheme, electron carriers known to function in electron flow from H₂O to NADP⁺ are arranged vertically at their midpoint redox potential.

Mechanisms of Electron Transport

• Almost all of the chemical processes that make up the light reactions of photosynthesis are carried out by four major protein complexes:
  – PSII
  – The cytochrome b₆f complex
  – PSI
  – ATP synthase
**Z-Scheme**

What are the functions of the four major protein complexes?

1. PSII oxidizes H₂O to O₂ in the thylakoid lumen; in the process protons are released into the lumen.

2. Cytochrome *b₆f* receives electrons from PSII and delivers them to PSI.

3. PSI reduces NADP⁺ to NADPH in the stroma by the action of ferredoxin (Fd) and the flavoprotein ferredoxin NADP reductase.

4. Cytochrome *b₆f* complex also pumps protons into the lumen, contributing to the electrochemical proton gradient.

5. ATP synthase produces ATP as protons diffuse back through it from the lumen into the stroma.

**Z-Scheme**

• Vertical arrows represent photon absorption by reaction center chlorophylls
  – P₆₈₀ for PSII
  – P₇₀₀ for PSI
• The excited PSII reaction center chlorophyll, P680*, transfers an electron to pheophytin (Pheo).

• On the oxidizing side of PSII (to the left of the arrow joining P680) with P680*, P680 oxidized by light is re-reduced by YZ, which received electrons from oxidation of water.

• On the reducing side of PSII (to the right of the arrow joining P680 with P680*), pheophytin transfers electrons to the acceptor QA and QB, which are plastoquinones.
The cytochrome \textit{b}_{6f} transfers electrons to plastocyanin, a soluble protein.

Plastocyanin in turn reduces P700$^+$ (oxidized P700).

The acceptor of electrons from P700*, known as (A$_0$), is thought to be a chlorophyll, and the next acceptor (A$_1$) is a quinone.

A series of membrane-bound iron-sulfur protein (Fe$_{S_3}$, Fe$_{S_4}$, and Fe$_{S_5}$) transfer electrons to soluble ferredoxin (Fd).
Z-Scheme

• The soluble flavoprotein ferredoxin-NADP reductase (FNR) reduces NADP⁺ to NADPH.
• NADPH is used in the Calvin cycle to reduce CO₂.

Transfer of Electrons and Protons in the Thylakoid Membranes

• Water is oxidized and protons are released in the lumen by PSII.
• PSI reduces NADP⁺ to NADPH in the stroma via the action of ferredoxin (Fd) and the flavoprotein ferredoxin-NADP reductase (FNR).

• Cytochrome b_{6f} complex also transports protons into the lumen, contributing to the electrochemical proton gradient.
Transfer of Electrons and Protons in the Thylakoid Membranes

- Protons in the lumen must diffuse to the ATP synthase enzymes, where their diffusion down the electrochemical gradient is used to synthesize ATP in the stroma.

Transfer of Electrons and Protons in the Thylakoid Membranes

- Reduced plastoquinone (PQH₂) and plastocyanin transfer electrons to cytochrome b₆f and to PSI, respectively.
- Dashed lines represent electron transfer; solid lines represent proton movement.

SUMMATION

- Water is oxidized to oxygen by PSII.
- Pheophytin and two quinones accept electrons from PSII.
- Electron flow through the cytochrome b₆f complex also transports protons.
Summation (cont.)

- Plastoquinone and plastocyanin carry electrons between PSII and PSI.

- The PSI reaction center reduces NADP⁺ to NADPH via the action of ferredoxin and the flavoprotein ferredoxin-NADP reductase.

Cyclic versus Noncyclic Photophosphorylation

- Cyclic → electrons are transferred from P700*, the excited reaction center of PSI, to ferredoxin and then to the cytochrome b₆f complex, rather than NADP⁺.

- Protons are pumped by this complex as electrons return to the reaction center through plastocyanin.

- Cyclic electron flow is coupled to proton pumping into the lumen.

- ATP is generated without the concomitant formation of NADPH.

Some Herbicides Block Electron Flow

- Herbicides such as DCMU (dichlorophenyl-dimethylurea), known as diuron, and methyl viologen, known as paraquat, block photosynthetic electron flow.

- Diuron acts by blocking electron flow at the quinone acceptor of PSII.

- Competes for the binding site of plastoquinone normally occupied by Qₒ.
Some Herbicides Block Electron Flow

• Paraquat acts by accepting electrons from the early acceptors of PSI and then reacting with \( \text{O}_2 \) to form superoxide, \( \text{O}_2^- \), a species that is very damaging to chloroplast components, especially lipids.

Proton Transport And ATP Synthesis in the Chloroplast

• Another fraction of the captured light energy in chloroplasts is used for light-dependent ATP synthesis, which is known as photophosphorylation.

• Photophosphorylation requires electron flow, although under some conditions, electron flow and photophosphorylation take place independently of each other.

• Electron flow without accompanying phosphorylation is said to be uncoupled.

How Does Photophosphorylation Work?

• Via the chemiosmotic mechanism.

• Basic principle → ion concentration difference and electric-potential differences across membranes are a source of free energy that can be utilized by the cell.

• Second law of thermodynamics describes that any nonuniform distribution of matter or energy represents a source of energy.

• The oxidation of water is the primary source of electrons.
**How Does Photophosphorylation Work?**

- The asymmetric nature of the photosynthetic membrane and the fact that proton flow from one side to the other of the membrane accompanies electron flow has been previously mentioned.

- As a result of electron transport, the direction of proton translocation is such that:
  - The stroma becomes more alkaline (fewer H⁺ ions)
  - The lumen becomes more acidic (more H⁺ ions)

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**Evidence Supporting Chemiosmotic Mechanism**

- Jagendorf et al. → Chloroplast thylakoids kept previously at pH 8.0 were equilibrated in an acid medium at pH 4.0.

- Thylakoids were then transferred to a pH 8.0 buffer containing ADP and P₇.

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**Evidence Supporting Chemiosmotic Mechanism**

- The proton gradient generated by this manipulation provided a driving force for ATP synthesis in absence of light.

- Experiment verified prediction of the chemiosmotic theory that a chemical potential across a membrane can provide energy for ATP synthesis.
Repair and Regulation of the Photosynthetic Machinery

- Photosynthetic machinery vulnerable to damage at the molecular level.

- Photosynthetic systems are designed to absorb large amounts of light energy and process it into chemical energy.

- Energy in a photon can be damaging at the molecular level, especially under unfavorable conditions (i.e. excess light, heat).

- In excess, light energy can lead to the production of the toxic species, such as superoxide, singlet oxygen, and peroxide – leads to damage of membranes if light energy is not dissipated safely.

- Complex regulatory and repair mechanisms exist:
  - Protective and Scavenging
    - Regulation of energy flow in the antenna system, to avoid excess excitation of the reaction centers to ensure that the two photosystems are equally driven.

- In addition to protective and scavenging mechanisms, additional mechanisms are required to repair the system.
  - Carotenoids, in addition to their role as accessory pigments, play an essential role in photoprotection.
Repair and Regulation of the Photosynthetic Machinery

- Why is a photoprotection mechanism needed?
  - Photosynthetic membranes can be easily damaged by large amounts of energy absorbed by pigments if this energy cannot be stored by photochemistry.
  - Photoprotection mechanism can be visualized as a safety valve—vents excess energy before organism is damaged.

Carotenoids As Photoprotective Agents

- When energy stored in chlorophylls in the excited state is rapidly dissipated by excitation transfer or photochemistry the excited state is said to be quenched.
- If the excited state of chlorophyll is not rapidly quenched, it can react with molecular oxygen to form an excited state of oxygen known as singlet oxygen ($^{1}\text{O}_2$).
- These extremely reactive singlet oxygen molecules react with and damage many cellular components, especially lipids.

Carotenoids As Photoprotective Agents

- Finally, the punch line. Carotenoids exert their photoprotective action by rapidly quenching the excited state of chlorophyll.
- The excited state of carotenoids does not have sufficient energy to form singlet oxygen, so it decays back to its ground level while losing energy as heat.
- Recently, carotenoids were found to play a role in nonphotochemical quenching, which is another protective and regulatory mechanism.
**Photoinhibition**

- The inhibition of photosynthesis by excess light.
- A major factor in stability of the photosynthetic apparatus.
- Occurs when excess excitation arriving at the PSII reaction center leads to its inactivation and damage.

**Photoinhibition**

- Reversible in early stages, but under prolonged inhibition leads to damage of D1 protein which makes up part of PSII reaction center complex.
- The D1 protein can be removed from the membrane and replaced with a newly synthesized molecule.

**Oxygen-Evolving Complex (OEC)**

- Introduction:

  PS II
  - antenna contains equal amounts of chlorophyll a and chlorophyll b
  PS I
  - high ratio of chlorophyll a to chlorophyll b

  PS I and PS II work in tandem to catalyze the light-driven movement of electrons from H₂O to NADP⁺.
Oxygen-Evolving Complex (OEC)

- Plastocyanin is the soluble protein that carries electrons between the two photosystems.
- To raise the energy of electrons derived from H₂O to the energy level required to reduce NADP⁺ to NADPH, each electron must be lifted twice (heavy arrows) by photons absorbed in PS I and PS II.
- One photon is required per electron in each photosystem.

Oxygen-Evolving Complex (OEC)

- Also known as water-splitting complex.
- The ultimate source of electrons passed to NADPH in plant (oxygenic) photosynthesis is water.
- The excited PS II reaction center chlorophyll (P680⁺), after giving up an electron to pheophytin, must acquire an electron to return to its ground state (P680⁺⁺) in order to be able to capture another photon.

Oxygen-Evolving Complex (OEC)

- About 3 billion years ago, evolution of primitive photosynthetic bacteria produced a photosystem capable of taking electrons from a donor that is always available—water.
- In this process, two water molecules are split, yielding four electrons, four protons, and molecular oxygen.

\[ 2 \text{H}_2\text{O} \rightarrow 4 \text{H}^+ + 4 \text{e}^- + \text{O}_2 \]
A single photon of visible light does not have enough energy to break the bonds in water. Four photons are required for this photolytic cleavage reaction.

Where does the oxygen-evolving complex come into play?

The four electrons abstracted from water do not pass directly to P680⁺, which can accept only one electron at a time.

The oxygen-evolving complex (also called the water-splitting complex), instead passes four electrons one at a time to P680.

The immediate electron donor to P680⁺ is a Tyr residue (often designated Z or Tyr; or Y₂ (Taiz and Zeiger) in protein subunit D1 of the PS II reaction center.

The tyrosine molecule loses both a proton and an electron, generating the electrically neutral Tyr free radical, Tyr

\[ 4 \text{P680}^+ + 4 \text{Tyr} \rightarrow 4 \text{P680} + 4 \text{Tyr} \]

The Tyr radical regains its missing electron and proton by oxidizing a cluster of four manganese ions in the water-splitting complex.
**Oxygen-Evolving Complex (OEC)**

- With each single electron transfer, the Mn cluster becomes more oxidized; four single-electron transfers, each corresponding to the absorption of one photon, produce a charge of +4 on the Mn complex.

\[
4 \text{Tyr} + [\text{Mn complex}]^0 \rightarrow 4 \text{Tyr} + [\text{Mn complex}]^{4+}
\]

**Oxygen-Evolving Complex (OEC)**

- In this stage, the Mn complex can take four electrons from a pair of water molecules, releasing 4H\(^+\) and O\(_2\):  

\[
[\text{Mn complex}]^{4+} + 2 \text{H}_2\text{O} \rightarrow [\text{Mn complex}]^0 + 4 \text{H}^+ + \text{O}_2
\]

Because the four protons produced in this reaction are released into the thylakoid lumen, the OEC acts as a proton pump, driven by electron transfer.