

Photosynthesis

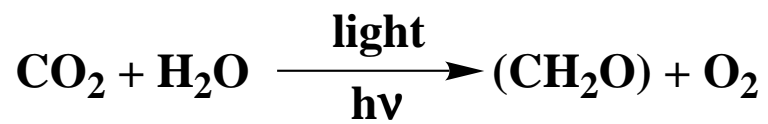
Chloroplasts

Light Reactions

(photons \rightarrow NADPH + ATP)

Dark Reactions

(CO₂ + H₂O \rightarrow carbohydrate)



Chloroplasts

Site of photosynthesis in algae and higher plants

1 to 1000 per cell

typically ~5- μm long ellipsoids

Outer membrane

permeable

Intermembrane space

Inner membrane

impermeable

Stroma

similar to mitochondrial matrix

Dark reaction enzymes (Calvin cycle or reductive pentose phosphate cycle), DNA, RNA, ribosomes

Thylakoid

highly folded vesicle arising from invaginations of inner membrane (similar to mitochondrial cristae)

electron transport chain proteins

10 to 100 grana (disc-like sacs) interconnected by stroma lamellae

10% phospholipids

80% uncharged mono- and digalactosyl diacylglycerols

10% sulfoquinovosyl diacylglycerols (sulfolipids)

Light Reactions

Absorption of light

Chlorophyll (Chl and bacteriochlorophyll, BChl)

differs from heme:

Mg²⁺

cyclopentanone ring V fused to pyrrole ring III

pyrrole ring IV partially reduced in Chl *a* and Chl *b* (rings

II and IV partially reduced in BChl *a* and BChl *b*)

propionyl side chain of ring IV esterified

Light Reactions

Absorption of light

Plank's law:

$$E = h\nu = \frac{hc}{\lambda}$$

h = Plank's constant = 6.626×10^{-34} J·s

c = speed of light = 2.998×10^8 m·s⁻¹ (vacuum)

λ = wavelength

Molecules absorb photons whose energy match the energy difference between ground and excited states

Beer-Lambert law:

$$A = \log \frac{I_0}{I} = \epsilon cl$$

A = absorbance

I_0 and I = incident and transmitted intensities

ϵ = molar extinction coefficient

c = molar concentration

l = sample pathlength in cm

Light Reactions

Absorption of light

Internal conversion - electronic energy converted to heat, time frame $<10^{-11}$ s

Fluorescence - excited state decays to ground state by emitting photon, time frame $\sim 10^{-8}$ s

Exciton transfer (resonance energy transfer) - excited molecule transfers its excitation energy to nearby unexcited molecules, important in funneling light energy to photosynthetic reaction centers

Photooxidation - light-excited donor molecule transfers an electron to an acceptor molecule, the oxidized donor relaxes to ground state by oxidizing some other molecule

Light Reactions

Absorption of light

Chlorophylls (**light-harvesting antennas**) function to gather photons and transfer energy by exciton transfer to a photosynthetic reaction center, time frame $<10^{-10}$ s with 90% efficiency

Light-harvesting complex-II (LHC-II)

most abundant membrane protein in chloroplasts of green plants

232-residue transmembrane protein

at least 7 Chl *a*, 5 Chl *b* and 2 carotenoids

function to gather light energy and prevent energy transfer to O₂

Phycobilisomes (algae and cyanobacteria)

phycobiliproteins

phycocyanobilin pigment

phycoerythrobilin pigment

bound to outer face of photosynthetic membrane

Light Reactions

Electron transport in photosynthetic bacteria

Reaction center composition:

transmembrane protein, 3 subunits (H, L, M)

P870 (BChl *a*)

4 BChl *b* (two form a "special pair")

bacteriopheophytin *b* (BPheo *b*, 2H⁺ replace Mg²⁺)

nonheme/non-Fe-S Fe²⁺

ubiquinone

menaquinone

Photooxidation of P870 "special pair" → BPheo *a* →
menaquinone → ubiquinone → Q cycle → cyt *bc*₁ (similar
to Complex III) → cyt *c*₂ → 4 heme cyt *c* → P870⁺

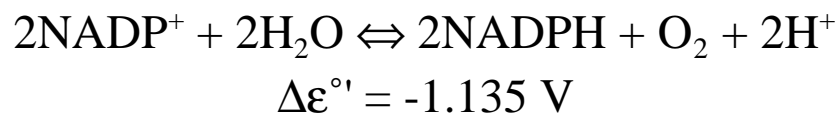
Sequence of electron transfers has a quantum yield of
~100%!

No net oxidation-reduction, functions to translocate H⁺s
across plasma membrane and photophosphorylation
produces ATP, both processes similar to those discussed in
oxidative phosphorylation

Light Reactions

Two-center electron transport (plants and cyanobacteria)

Reducing power of photooxidation of H₂O drives NADPH production



$$\Delta G^{\circ'} = +438 \text{ kJ}\cdot\text{mol}^{-1}$$

(requires 1 einstein of 223-nm photons)

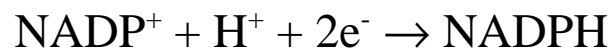
This means that more than one photon of visible light is required for production of one O₂ molecule (8 to 10 photons)

Light Reactions

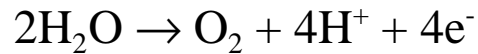
Two-center electron transport

Photosystems I and II (Z-scheme)

Photosystem I (PSI) - generates strong reductant (weak oxidant)



Photosystem II (PSII) - generates strong oxidant (weak reductant)



Cytochrome *b₆f* connects PSII and PSI

Light Reactions

Two-center electron transport

Three thylakoid transmembrane protein complexes

1. **PSII - P680** (Chl *a*), Pheo *a*, Q_A, Q_B (herbicides complete with Q)
2. **Cytochrome *b₆f* complex** - (resembles cyt *bc₁* and Complex III) cyt *f*, cyt *b₆* (2 hemes), [2Fe-2S] protein, bound plastoquinol, transport H⁺ from outside to inside of thylakoid membrane using Q cycle
Generates much of the H⁺ gradient that drives ATP synthesis
3. **PSI** - 7 subunits (2 large, 5 small), P700, 100 to 200 Chl *a*, 12 to 16 **β-carotene**, 3 [4Fe-4S] clusters, 2 **phylloquinone** (vit K₁)

Intercomplex electron transfer between PSII and cyt *b₆f* by **plastoquinone/plastoquinol (Q/QH₂)**

Intercomplex electron transfer between cyt *b₆f* and PSI by **plastocyanin (PC)**, a peripheral membrane Cu metalloprotein)

Oxygen evolving complex (OEC) - protein complex with 4 Mn (III/IV)-O²⁻ clusters, 2 or 3 Ca²⁺, and 4 or 5 Cl⁻ ions

Z - tyrosine radical-containing (macro)molecule

Light Reactions

Two-center electron transport

Electrons from PSI may follow two routes:

1. **Noncyclic pathway** to 11-kDa, [2Fe-2S] soluble **ferredoxin (Fd)**, $1e^-$ donor/acceptor) located in stroma, where **FAD-containing ferredoxin-NADP⁺ reductase (FNR)** reduces NADP⁺
2. **Cyclic pathway** to return to plastoquinone (Q) pool, translocates H⁺s across thylakoid membrane, independent of PSII

Functions to increase ATP production relative to that of NADPH

PSI resembles (functionally and genetically) bacterial photosystems

Light Reactions

Two-center electron transport

Distribution of photosynthetic complexes

PSI occurs **mainly in unstacked stroma lamellae**, in contact with stroma, where it has access to NADP⁺

PSII occurs **almost exclusively between stacked grana**, out of direct contact with stroma

Cytochrome *b_f* is **uniformly distributed throughout membrane**

Why?

Need to separate PSII from PSI so that exciton transfer does not occur

Allows response to low (long wavelength) and high (short wavelength) light illumination (light-activated protein kinase-dependent feedback mechanism)

Light Reactions

Photophosphorylation

Chloroplasts couple dissipation of H^+ gradient to synthesis of ATP (resembles mitochondrial system)

CF_1CF_0 complex

1. CF_0 is hydrophobic transmembrane protein, H^+ translocating channel
2. CF_1 is hydrophilic peripheral membrane protein, $\alpha_3\beta_3\gamma\delta\varepsilon$ composition, β is reversible ATPase, γ controls H^+ flow from CF_0 to CF_1
3. **Inhibited** by **oligomycin** and **dicyclohexylcarbodiimide (DCCD)**

Chloroplast ATP synthase is **located in unstacked portions of thylakoid membrane** in contact with stroma

Translocates H^+ out of thylakoid space

How does that compare to the mitochondrial ATP synthase?

Light Reactions

Photophosphorylation

At saturating light intensities, chloroplasts generate proton gradient of ~3.5 pH units, which arise from two sources

1. Evolution of O_2 releases $4H^+$ (from stroma by way of NADPH synthesis) into thylakoid space
2. Transport of e^- s through *cyt b_6/f* translocates $8H^+$ (from stroma to thylakoid space)

~12 H^+ translocated per O_2 produced by noncyclic electron transport

Thylakoid membrane allows passage of Mg^{2+} and Cl^- , which results in elimination of membrane potential ($\Delta\Psi$)

Electrochemical gradient is almost entirely pH gradient

ATP production:

Noncyclic electron transport

4 ATP per O_2 evolved (and 2 NADPH \rightarrow 6 ATP)

0.5 ATP per photon absorbed

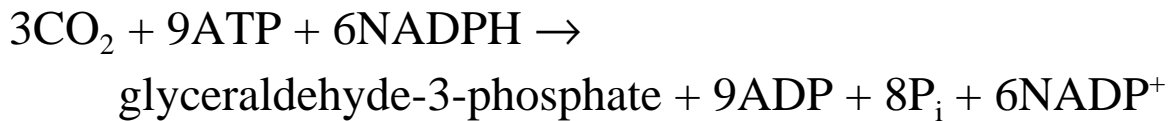
so 1.25 ATP per photon absorbed!

Cyclic electron transport

0.67 ATP per photon absorbed

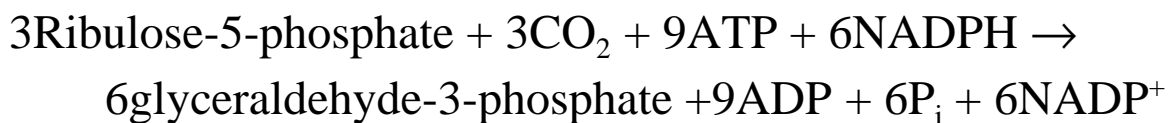
Dark Reactions

The Calvin cycle (reductive pentose phosphate cycle)



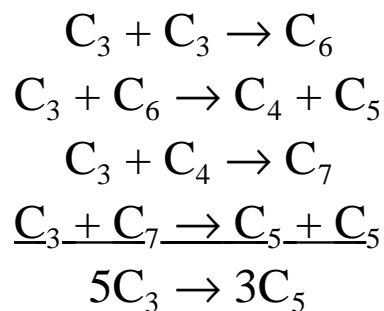
Two-stage process:

1. Production phase



one glyceraldehyde-3-phosphate \rightarrow biosynthesis

2. Recovery phase



Dark Reactions

The Calvin cycle

Uses enzymes from glycolytic, gluconeogenic, and pentose phosphate pathways

Three unique enzymes:

phosphoribulokinase

ribulose biphosphate carboxylase (RuBP carboxylase)

sedoheptulose biphosphatase (SBPase)

Dark Reactions

Ribulose biphosphate carboxylase (RuBP carboxylase)

The most abundant protein in the biosphere!

$$k_{\text{cat}} \sim 3 \text{ s}^{-1}$$

Eight large (L) subunits (477 residues, encoded by chloroplast DNA) - catalytic site

Eight small subunits (123 residues, specified by nuclear gene) - unknown function

L₈S₈ composition

rate-determining step is C3 H⁺ abstraction to generate enediolate

requires Mg²⁺

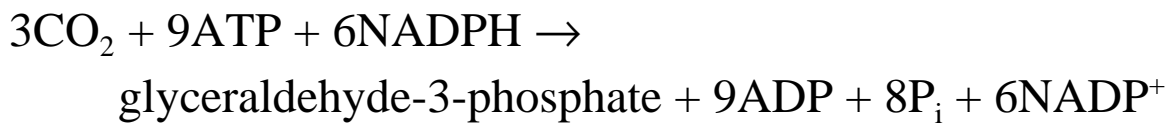
$$\Delta G^{\circ} = -35.1 \text{ kJ}\cdot\text{mol}^{-1}$$

ribulose-biphosphate carboxylase activase - catalyzes carbamate formation from CO₂ and ε-amino of Lys residue

Dark Reactions

The Calvin cycle

Recall stoichiometry of Calvin cycle:



Glyceraldehyde-3-phosphate may be converted to glucose-1-phosphate

Precursor of higher carbohydrates (through nucleotide sugars):

sucrose (major transport sugar)

starch (major storage polysaccharide)

cellulose (primary structural polysaccharide)

precursor to fatty acids and amino acids

Dark Reactions

Control of Calvin cycle

During the day, plants use photosynthesis to produce ATP and NADPH for use in Calvin cycle

At night, plants process nutritional stores to produce ATP and NADPH

Must have light **activate Calvin cycle** and **deactivate glycolysis** (prevent futile cycle)

Regulation of **RuBP carboxylase**, **FBPase**, and **SBPase** by light-dependent factors

RuBP carboxylase:

photons increase stroma **pH** from 7 to 8 (pH optimum for enzyme)

photons translocate H^+ to thylakoid, which drives **Mg²⁺** efflux to stroma

dark reaction intermediate (**2-carboxyarabinitol-1-phosphate, CA1P**) **inhibits** RuBP carboxylase

Dark Reactions

Control of Calvin cycle

FBPase and SBPase:

activated by increased pH, Mg^{2+} , and NADPH

activated by reduced thioredoxin (12 kDa redox sensitive protein), which is regulated by ferredoxin-thioredoxin reductase

Light stimulates Calvin cycle and deactivates glycolysis

Absence of light stimulates glycolysis and deactivates Calvin cycle

Dark Reactions

Photorespiration and the C₄ cycle

Photorespiration occurs at high [O₂] with production of CO₂

RuBP carboxylase has two substrates - CO₂ and O₂!

Ribulose biphosphate carboxylase-oxygenase (Rubisco)

Ribulose-5-phosphate + O₂ →
3-phosphoglycerate + 2-phosphoglycolate

Dark Reactions

Photorespiration and the C_4 cycle

CO_2 results from series of peroxisomal and mitochondrial reactions:

phosphoglycolate phosphatase

glycolate oxygenase

catalase - heme enzyme takes $H_2O_2 \rightarrow O_2 + H_2O$

transamination reactions

hydroxypyruvate reductase

glycerate kinase

Net result is that some photosynthetically generated ATP and NADPH is dissipated!

May be useful in protecting cells when CO_2 levels are low

Dark Reactions

Photorespiration and the C₄ cycle

CO₂ compensation point - [CO₂] at which rates of photosynthesis and photorespiration are equal

For many plants, CO₂ compensation point is ~40 to 70 ppm (normal atmospheric [CO₂] is 330 ppm)

so, photosynthesis > photorespiration rates

But this is temperature dependent!

On hot sunny days, CO₂ is depleted and O₂ is elevated at the chloroplast, photosynthesis ~ photorespiration!

Lessening oxygenase activity of Rubisco would be potentially beneficial

Dark Reactions

Photorespiration and the C₄ cycle

C₄ plants (sugar cane, corn, weeds) concentrate CO₂
CO₂ compensation point drops to ~ 2 to 5 ppm

C₄ cycle:

characteristic anatomy (**bundle-sheath cells, mesophyll cells**)

2 ATP to concentrate CO₂ in bundle-sheath cells

Photosynthesis in C₄ plants consumes 5 ATP per CO₂ fixed
(compare to 3 ATP for Calvin cycle alone)

C₄ plants occur largely in tropical areas (high temperatures and light intensity)

C₃ plants occur largely in cooler areas (lower light intensity)

Desert plants use **Crassulacean acid metabolism (CAM)** to minimize H₂O loss during the day and maximize CO₂ absorption at night

Large amounts of phosphoenolpyruvate are required from starch breakdown and glycolysis

Malate is used to produce CO₂ for the Calvin cycle

Pyruvate is used to resynthesize starch