Photosynthesis

Chloroplasts

Light Reactions
(photons $\rightarrow$ NADPH + ATP)

Dark Reactions
($CO_2 + H_2O \rightarrow$ carbohydrate)

$$\mathbf{CO_2 + H_2O \xrightarrow[h\nu]{light} (CH_2O) + O_2}$$

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:
Mg^{2+}
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:

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

h = Plank's constant =6.626 x 10^{-34} J·s
c = speed of light = 2.998 x 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:

$$A = \log \frac{I_0}{I} = \varepsilon c l$$

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 $\sim10^{-8}$ 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 O_2

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 Mg^{2+})
nonheme/non-Fe-S Fe^{2+}
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 H_2O drives NADPH production

$$O_2 + 4e^- + 4H^+ \Leftrightarrow 2H_2O \quad \varepsilon^{\circ\prime} = +0.815 \text{ V}$$

$$NADP^+ + H^+ + 2e^- \Leftrightarrow NADPH \qquad \varepsilon^{\circ\prime} = -0.320 \text{ V}$$

$$2NADP^+ + 2H_2O \Leftrightarrow 2NADPH + O_2 + 2H^+$$
$$\Delta\varepsilon^{\circ\prime} = -1.135 \text{ V}$$

$$\Delta G^{\circ\prime} = +438 \text{ kJ}\cdot\text{mol}^{-1}$$
(requires 1 einstein of 223-nm photons)

This means that more than one photon of visible light is required for production of one O_2 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)

$$NADP^+ + H^+ + 2e^- \rightarrow NADPH$$

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

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$$

Cytochrome b_6f 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 b_6f complex - (resembles cyt bc_1 and Complex III) cyt *f*, cyt b_6 (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 K_1)

Intercomplex electron transfer between PSII and cyt b_6f by plastoquinone/plastoquinol (Q/QH_2)

Intercomplex electron transfer between cyt b_6f and PSI by plastocyanin (PC, a peripheral membrane Cu metalloprotein)

Oxygen evolving complex (OEC) - protein complex with 4 Mn (III/IV)-O^{2-} clusters, 2 or 3 Ca^{2+}, 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 $NAPD^+$

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_6f 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_6f 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)

$3CO_2 + 9ATP + 6NADPH \rightarrow$
glyceraldehyde-3-phosphate + $9ADP + 8P_i + 6NADP^+$

Two-stage process:

1. Production phase

$3Ribulose\text{-}5\text{-}phosphate + 3CO_2 + 9ATP + 6NADPH \rightarrow$
$6glyceraldehyde\text{-}3\text{-}phosphate + 9ADP + 6P_i + 6NADP^+$

one glyceraldehyde-3-phosphate → biosynthesis

2. Recovery phase

$5Glyceraldehyde\text{-}3\text{-}phosphate \rightarrow 3ribulose\text{-}5\text{-}phosphate$

$$C_3 + C_3 \rightarrow C_6$$
$$C_3 + C_6 \rightarrow C_4 + C_5$$
$$C_3 + C_4 \rightarrow C_7$$
$$\underline{C_3 + C_7 \rightarrow C_5 + C_5}$$
$$5C_3 \rightarrow 3C_5$$

Dark Reactions

The Calvin cycle

Uses enzymes from glycolytic, gluconeogenic, and pentose phosphate pathways

Three unique enzymes:

phosphoribulokinase
ribulose bisphosphate carboxylase (RuBP carboxylase)
sedoheptulose bisphosphatase (SBPase)

Dark Reactions

Ribulose bisphosphate carboxylase (RuBP carboxylase)

The most abundant protein in the biosphere!

k_{cat} ~3 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

L_8S_8 composition

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

requires Mg^{2+}

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

ribulose-bisphosphate carboxylase activase - catalyzes carbamate formation from CO_2 and ε-amino of Lys residue

Dark Reactions

The Calvin cycle

Recall stoichiometry of Calvin cycle:

$3CO_2 + 9ATP + 6NADPH \rightarrow$
glyceraldehyde-3-phosphate + $9ADP + 8P_i + 6NADP^+$

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 Mg^{2+} 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 C_4 cycle

Photorespiration occurs at high [O_2] with production of CO_2

RuBP carboxylase has two substrates - CO_2 and O_2!

Ribulose bisphosphate carboxylase-oxygenase (Rubisco)

Ribulose-5-phosphate + O_2 $\rightarrow$

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 C_4 cycle

CO_2 compensation point - [CO_2] at which rates of photosynthesis and photorespiration are equal

For many plants, CO_2 compensation point is ~40 to 70 ppm (normal atmospheric [CO_2] is 330 ppm)

so, photosynthesis > photorespiration rates

But this is temperature dependent!

On hot sunny days, CO_2 is depleted and O_2 is elevated at the chloroplast, photosynthesis ~ photorespiration!

Lessening oxygenase activity of Rubisco would be potentially beneficial

Dark Reactions

Photorespiration and the C_4 cycle

C_4 plants (sugar cane, corn, weeds) concentrate CO_2
CO_2 compensation point drops to ~ 2 to 5 ppm

C_4 cycle:

characteristic anatomy (bundle-sheath cells, mesophyll cells)
2 ATP to concentrate CO_2 in bundle-sheath cells

Photosynthesis in C_4 plants consumes 5 ATP per CO_2 fixed (compare to 3 ATP for Calvin cycle alone)

C_4 plants occur largely in tropical areas (high temperatures and light intensity)
C_3 plants occur largely in cooler areas (lower light intensity)

Desert plants use Crassulacean acid metabolism (CAM) to minimize H_2O loss during the day and maximize CO_2 absorption at night

Large amounts of phosphoenolpyruvate are required from starch breakdown and glycolysis

Malate is used to produce CO_2 for the Calvin cycle

Pyruvate is used to resynthesize starch