The Dynamics of Thylakoid Membranes from Higher Plants

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Photosynthesis – a global perspective

Takes place almost everywhere on Earth where green plants, algae, and photosynthetic bacteria can be found.
Photosynthesis – a global perspective

- Energy for photosynthesis comes from sun light
- Two sets of reactions – light dependent and light independent
- Affected by temperature, light intensity/quality and CO$_2$ level
Photosynthesis – a global perspective

- Ultimate energy source for living organisms – all food and oxygen in Earth’s biosphere arrive from photosynthesis

- Source for all fossil fuel reserves – products of photosynthesis were converted into fuels over millions of years

- One tree makes 12 kg of biomass and outputs 9400 L of oxygen in 24 h – enough for family of FIVE!
Photosynthesis – where it all takes place

Tree | Leaf | Plant cell

Thylakoid membrane | Chloroplasts

Outer and inner membranes
Intermembrane space
Stroma
Photosynthetic membranes

Photosynthetic bacteria
*Rhodopseudomonas viridis*

Cyanobacteria
*Synechocystis sp. PCC 6803*

Marine cyanobacteria
*Prochlorococcus marinum*

Green alga
*Chlamydomonas reinhardtii*

Chloroplast
*Spinacia oleracia L.*
Domains of the thylakoid membrane from higher plants
Thylakoid membrane complexes and electron/proton transfer reactions
Photosystem I

- Light driven plastocianine ferredoxine oxidoreductase
- Electron transfer reactions
- Analogous to green sulphur and hellobacteria (iron sulfur type reaction center)
- ~ 300 kDa, about 15 protein subunits
- Trimer in cyanobacteria, monomer in higher plants
- Crystal structure is solved to 2.0 Å resolution
- Branched electron transfer is debated
Cytochrome b$_6$f complex

- Plastoquinone plastocyanine oxidoreductase
- Electron and proton transfer reactions
- Q cycle to translocate proton through the membrane
- Found in the dimeric form
- Analogous to Cytochrome bc$_1$ complex in photosynthetic bacteria and mitochondria
- Crystal structure is solved to the 3.0 Å resolution
- A single Chl molecule is found; function is unknown
Photosystem II

- Light driven water plastoquinone oxidoreductase. Can split water and $O_2$ is released as a byproduct, turnover rate is about 100 molecules per second
- Electron and proton transfer reactions
- Analogous to purple bacteria (quinone type reaction center)
- ~ 900 kDa, more than 25 protein subunits, structurally highly heterogenic
- Operates at highly oxidizing potentials
- Crystal structure is solved to the medium 3.0 Å resolution
- Water oxidation mechanism is unknown
The catalytic site of Photosystem II
CaMn$_4$ cluster and the S-state cycle

Oxygen release pattern: the S state cycle
Distribution of Photosystems in the thylakoid membrane from higher plants

Grana

PSII dimer

PSI

Cytb$_6$f

LHCII

PSII monomer

ATP-synthase

Stroma lamellae
Methods to study photosynthetic complexes : Biochemistry

• Separation of different parts of the thylakoid membrane (different domains) without disturbing their native composition

• Isolation and purification of the photosynthetic membranes and complexes on the different levels – chloroplasts, thylakoid membranes, PSI or PSII membranes, PSI and PSII core complexes, reaction centers etc. from plants, green algae and cyanobacteria

• Supramolecular and protein composition analysis of different complexes in the thylakoid membrane
Methods to study photosynthetic complexes: Biochemistry

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Activity (i.e. PSII oxygen evolution, μmol O₂ / mg Chl h)</th>
<th>EPR signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells, chloroplasts</td>
<td>~ 80</td>
<td></td>
</tr>
<tr>
<td>Thylakoid membranes</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>PSII membranes</td>
<td>500 - 700</td>
<td></td>
</tr>
<tr>
<td>PSII core reparations</td>
<td>~ 5000</td>
<td></td>
</tr>
<tr>
<td>Reaction Center preparations</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
Methods to study photosynthetic complexes: Biophysics and Spectroscopy

• Electron and proton transport measurements

• Optical and fluorescence spectroscopy; time resolved measurements

• EPR spectroscopy – conventional and advanced (pulse) methods

• Application of the short (ns) laser flashes to study different intermediates of the catalytic meachanisms (i.e. S states)
Electron Paramagnetic Resonance (EPR) spectroscopy from PSII

- $g = 2.0046$
- $g = 1.69$
- $g = 1.8$
- $g = 3.01$
- $g = 2.96$
- $g = 0$

[Diagram showing various components and reactions related to electron transport and oxygen evolution.]
Electron Paramagnetic Resonance Spectroscopy on the S-states

$S_0$, $S_1$, $S_2$, $S_3$, $S_4$...

Tyr$_z^•$

e$\rightarrow$ e$\rightarrow$ e$\rightarrow$ e$\rightarrow$...
Photosystem II life cycle
photoinhabition / repair cycle

• Photosystem II is highly vulnerable to environmental stress

• Exhibit functional and structural heterogeneity and unevenly distributed in the thylakoid membrane

• Process several protective mechanisms such as energy dissipation in antenna, xanthophyll cycle, protein phosphorylation, state transition, etc

• Excess of light leads to inhibition of Photosystem II (photoinhibition). At the normal day light conditions every 30 min one Photosystem II is destroyed

• Reparation of Photosystem II is a complex process, which takes place in the different parts of the thylakoid membrane and requires the lateral movement of Photosystem II centers in the thylakoid membrane
How to study Repair process?

- Separation of the thylakoid domains and study of their biochemical and biophysical properties

- Application of the imaging technology – confocal fluorescence microscopy, EPR imaging, etc.

- Biogenesis of the photosynthetic complexes. In this case, the assembly and activation of the PSI, PSII or cyt b₆f complexes can be studied during greening of the etiolated plants

- Photoactivation experiments (assembly of the CaMn₄-cluster) (dark gron alga are an excellent model)
Understanding membrane dynamics – study of the thylakoid membrane domains

- Non-invasive, two phase separation of the different fractions of thylakoid membrane
- Biochemical and biophysical characterization of PSII, PSI and Cyt b₆f complex (antenna properties, protein composition, electron transfer reactions)
Isolation of the different membrane fractions
Two-phase separation technique

Stroma lamellae

Grana

Grana Margins

Grana Core

The end membrane (End of Grana) and the purified stroma lamellae (Y100) also can be separated
Characterization of Photosystem II in different fractions

<table>
<thead>
<tr>
<th>Domain</th>
<th>$O_2$ evolution ($\mu$mol/mg of Chl x h)</th>
<th>$O_2$ evolving centers (% of total PSII centers)</th>
<th>$Fv/Fo$</th>
<th>Chl a/b (mol/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grana Core</td>
<td>250-300</td>
<td>91</td>
<td>0.87-1.30</td>
<td>1.8-2.0</td>
</tr>
<tr>
<td>Grana</td>
<td>200-250</td>
<td>84</td>
<td>0.81-1.10</td>
<td>2.2-2.4</td>
</tr>
<tr>
<td>Margins</td>
<td>102</td>
<td>66</td>
<td>0.45-0.50</td>
<td>3.0-3.3</td>
</tr>
<tr>
<td>Stroma</td>
<td>80</td>
<td>43</td>
<td>0.27</td>
<td>4.5-5.0</td>
</tr>
<tr>
<td>Y100</td>
<td>0</td>
<td>0</td>
<td>0.20</td>
<td>6.0-6.7</td>
</tr>
<tr>
<td>Thylakoids</td>
<td>120</td>
<td>80</td>
<td>0.70</td>
<td>2.9</td>
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</table>
Thylakoid membrane domains
Quantification of PSI and PSII

EPR spectroscopy

Chemically or light oxidized sample

Dark adapted sample

TyrD• (1 spin/PSII center)

Difference spectra

P700+ (1 spin/PSI center)
Thylakoid membrane domains
Antenna properties

State transition phenomenon

77 K fluorescence spectra
Thylakoid membrane domains
Antenna properties

State transition phenomenon

77 K fluorescence spectra
Thylakoid membrane domains

Supramolecular composition of Photosystem II

A – PSII supercomplex
B – PSI, PSII dimers
C – ATPase
D – PSII monomer
E – Cytb$_{6}$ f dimer
F – LHCII trimer
G – Cytb$_{6}$ f monomer

BN-PAGE and the second dimension SDS-PAGE of different fractions from the thylakoid membrane

Immunoblot detection
Thylakoid membrane domains
Supramolecular composition of Photosystem II
Thylakoid membrane domains

Electron transport properties – EPR spectroscopy

<table>
<thead>
<tr>
<th>Domain of the thylakoid</th>
<th>Tyr$_D^{ox}$ %</th>
<th>Q$_A$-Fe$^{2+}$ %</th>
<th>S$_2$ State %</th>
<th>O$_2$ evolution %</th>
<th>Q$_A$-Fe$^{2+}$ signal</th>
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<tbody>
<tr>
<td>Grana Core</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Grana</td>
<td>82</td>
<td>94</td>
<td>92</td>
<td>81</td>
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<tr>
<td>Margin</td>
<td>59</td>
<td>39</td>
<td>40</td>
<td>37</td>
<td></td>
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<tr>
<td>Stroma</td>
<td>35</td>
<td>31</td>
<td>33</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Y100</td>
<td>15</td>
<td>13</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Thylakoid</td>
<td>66</td>
<td>70</td>
<td>81</td>
<td>43</td>
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</tr>
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</table>

S$_2$ state multiline

EPR measurements on different fractions

Magnetic field, G

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Electron transport properties – EPR spectroscopy

EPR measurements on different fractions

Magnetic field, G

---

Domain of the thylakoid

Tyr$_D^{ox}$ % Q$_A$-Fe$^{2+}$ % S$_2$ State % O$_2$ evolution % Q$_A$-Fe$^{2+}$ signal

Grana Core 100 100 100 100
Grana 82 94 92 81
Margin 59 39 40 37
Stroma 35 31 33 29
Y100 15 13 0 0
Thylakoid 66 70 81 43
Thylakoid membrane domains
Electron transport properties – Fluorescence

Flash-induced fluorescence decay in different fractions

<table>
<thead>
<tr>
<th>Domain</th>
<th>( Q_B ) binding, ms</th>
<th>Photoactivation, min</th>
<th>( Q_B ) binding, ms</th>
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<tbody>
<tr>
<td>Y100</td>
<td>29</td>
<td>Dark grown</td>
<td>32</td>
</tr>
<tr>
<td>Stroma</td>
<td>29</td>
<td>2</td>
<td>27</td>
</tr>
<tr>
<td>Margin</td>
<td>46</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>Grana</td>
<td>6.5</td>
<td>60</td>
<td>10</td>
</tr>
<tr>
<td>Grana Core</td>
<td>5.9</td>
<td>Light grown</td>
<td>8</td>
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<table>
<thead>
<tr>
<th>Domain</th>
<th>Recombination</th>
<th>Photoactivation, min</th>
<th>Recombination</th>
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<tbody>
<tr>
<td>Y100</td>
<td>39</td>
<td>Dark grown</td>
<td>90</td>
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<tr>
<td>Stroma</td>
<td>44</td>
<td>2</td>
<td>110</td>
</tr>
<tr>
<td>Margin</td>
<td>90</td>
<td>5</td>
<td>170</td>
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<td>---</td>
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<td>10</td>
<td>460</td>
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<td>---</td>
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<td>30</td>
<td>720</td>
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<tr>
<td>Grana</td>
<td>170</td>
<td>60</td>
<td>670</td>
</tr>
<tr>
<td>Grana Core</td>
<td>280</td>
<td>Light grown</td>
<td>930</td>
</tr>
</tbody>
</table>

+ DCMU
• Photosystem II migrates from the stroma lamellae to the grana during reparation process. Concomitantly with this lateral migration:

• The number of the Photosystem II centers is gradually increases (from 5 to 60% of the total amount)

• Supramolecular and protein composition is changing from the minimal monomeric protein unit to the fully assembled PSII supercomplexes

• Electron transport on both acceptor and donor side is activated leading to the fully competent centers
Special thanks to:
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Botanical Garden
View from Uppsala Castle