Chapter 1

INTRODUCTION

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1. Introduction

The process of photosynthesis involves the conversion of solar energy into a chemically stable form of energy. In higher plants and cyanobacteria water is the source of electrons for the light driven reduction of CO$_2$ to organic compounds and the overall reaction may be written as

$$2\text{H}_2\text{O} + \text{CO}_2 \xrightarrow{\text{hv, chloroplast}} \text{CH}_2\text{O} + \text{O}_2 + \text{H}_2\text{O}$$

The photosynthetic water oxidation is unique to plants and cyanobacteria. Photosynthetic bacteria use chemicals such as H$_2$S instead of water and thus do not produce oxygen as a by product.

The light energy is absorbed by light-harvesting pigment-protein complex and then transferred to the reaction centre where the primary photochemistry occurs. This results in charge separation at the reaction centres. In plants and cyanobacteria, this light driven oxidation reduction occurs in two photosystems containing the reaction centres P680 and P700. One of these photosystems, the photosystem II is the site of water oxidation, resulting in the release of molecular oxygen, protons and electrons. The electrons are transferred to photosystem I via the cyt b$_6$-f complex. Photosystem I provides the reducing power that eventually results in the reduction of NADP$^+$. The transfer of electrons from H$_2$O to NADP$^+$ is coupled to the accumulation of protons and the formation of an electrochemical gradient of H$^+$-ions across the membrane. The energy stored in this gradient is utilized by the coupling factor enzyme to synthesize ATP.

In higher plants the photosynthetic processes occur in the chloroplasts which consist of thylakoid membranes. The thylakoid membrane contains pigment-proteins and complexes of polypeptides which participate in the energy transduction processes.

2. Chloroplast Morphology

a) Arrangement of thylakoids

The thylakoid membrane forms an interconnected network of flattened vesicles which are organised into closely appressed or stacked membrane regions
called the grana thylakoid. These are interconnected with single, non-appressed or unstacked membranes called the stroma thylakoids (Fig.1.1). The space enclosed between the inner surface of the membrane is continuous between grana and stroma thylakoids (Staehelin and Arntzen, 1983).

The grana thylakoid stacking is mediated by a combination of van der Waals attraction forces and electrostatic repulsive forces between the negatively charged membranes (Barber, 1980; Sculley et al., 1980; Chow et al., 1980; Barber, 1982). The formation of grana stacks requires cation shielding of net negative membrane surface charge, as well as specific interactions among LHC polypeptides on opposing membranes (see Ryrie et al., 1980; Kaplan and Arntzen, 1982). The externally exposed positively charged polypeptide segment of the LHC, containing the amino-acids lysine and arginine, is necessary for the stabilization of membrane-membrane interactions and determines the reorganisation of charged groups along the membrane (Mullet et al., 1981). The organisation of LHC units and creation of domains with small or large negative surface charge (in grana or stroma thylakoids respectively) due to lateral displacement of protein complexes (see Barber, 1980), gives rise to enrichment of PS II in grana and PS I in the stroma region. The spatial segregation of PS II and PS I into separate membrane region influences the distribution of absorbed excitation energy among the two photosystems (Arntzen, 1978; Barber, 1980).

b) Structural properties and molecular organisation of thylakoid lipids

Photosynthetic membranes contain 35% (w/w) acyl lipids, of which 70% is contributed by the uncharged polyunsaturated glycolipids, monogalactosyl diacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG).

MGDG alone accounts for 40-50% of the total thylakoid lipids. However, it lacks the capacity to form a bilayer. Both MGDG and DGDG have extremely high polyunsaturated fatty acid content. The dominant galactolipid is dilinolenin (C18:3).

The remaining 30% of the thylakoid acyl lipids is made up of the anionic lipids diacylsulfoquinovosylglycerol (DSQ) and phosphatidyglycerol. A minor amount of the zwitterionic lipid phosphatidylcholine is also present.

The molecular shape of individual lipid molecules has been used to explain their physical behaviour in thylakoid membranes (Murphy, 1982; Sprague and Staehelin, 1983). It has been observed that the acyl lipids of thylakoid membranes
Fig. 1.1 Thin section through a portion of a spinach chloroplast illustrating interconnected stacked (grana; GT) and unstacked (stroma; ST) thylakoid membranes (X 80,000)
(Taken from Staehelin, 1986)
are asymmetrically distributed: (1) more acyl lipid is located on the inner than on the outer monolayer, (2) most of the MGDG and DSQ is on the inner monolayer, (3) DGDG may be evenly distributed across the membrane and (4) most of the PG is on the outer monolayer (Fig.1.2) (see Murphy, 1986).

Role of lipids in thylakoid membrane and effect of lipid hydrogenation (on PS II organisation) has been reported (Horvath et al., 1987).

c) Supramolecular organisation of thylakoids

The thylakoid membrane shows an asymmetrical arrangement of protein complexes between the two surfaces (inside and outside) of the membrane, where the specific biochemical functions occur. The functional asymmetry forms the basis of the overall activity of energy transduction (Mitchell, 1966). The organisation of chloroplast membrane components participating in the photosynthetic electron transport has been schematically represented in Fig.1.3. The supramolecular protein complexes of the thylakoid membrane include the photosystem II, the photosystem I, the cytochrome b₆-f complex and the H⁺-ATPase complex (see Fig.1.3). Each of them consist of several polypeptides. The two photosystems contain the chlorophylls and the accessory pigments (see Satoh, 1986). Less than 1% of the total chlorophylls of chloroplasts is bound with the reaction centre proteins and the rest (around 99%) of the chlorophylls serve as light harvesting pigment-protein complex (Staehelin and Arntzen, 1983). The electron transport between the various complexes in the thylakoid membrane is shown in Fig.1.4.

The spatial distribution of the various photosynthetic membrane components in the grana and stroma regions is summarised in Table 1.1.

d) Modifications in thylakoid membrane structure

i) Salt effect

Freeze fracture studies have revealed that in low salt-medium thylakoid membranes unstack and the lateral segregation of specific categories of intramembrane particles into grana and stroma membrane regions is lost (Ojakian and Satir, 1974; Staehelin, 1976). On resuspension in a high salt buffer the membrane gets restacked and complete resegregation of the particles takes place (Staehelin, 1976; Staehelin and Arntzen, 1979).

Deficiency of certain micronutrients (like Cu, B, Fe) is known to affect the electron transport.
Fig. 1.2 Schematic representation of thylakoid structure in cross-section. Packing constraints in highly curved margins favour the presence of cone shaped lipids (e.g. MGDG) in the inner, concave, monolayer and more wedge-shaped lipids on the outer convex monolayer.

(Taken from Murphy, 1986)
Fig. 1.3 The physical organisation of photosynthetic electron transport components in the thylakoid membrane of a green plant chloroplast 
(Taken from Steinback et al., 1985)
Fig. 1.4 Electron transport chain (Z scheme) in higher plants showing the two photosystems connected in series. The $E_m$ values of various components and the kinetics of electron transport have been taken from various sources, mentioned in the text.
Table 1.1 Spatial distribution of chloroplast membrane components

<table>
<thead>
<tr>
<th>Component</th>
<th>Stacked (grana) membranes</th>
<th>Unstacked (stroma) membranes</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS II</td>
<td>85</td>
<td>15</td>
<td>Andersson and Anderson (1980); Staehelin et al. (1977)</td>
</tr>
<tr>
<td>PS I</td>
<td>&lt; 15</td>
<td>&lt; 85</td>
<td>Andersson and Anderson (1980)</td>
</tr>
<tr>
<td>Cyt b₆/f</td>
<td>50</td>
<td>50</td>
<td>Cox and Andersson (1981); Anderson (1982); Allred and Staehelin (1985)</td>
</tr>
<tr>
<td>Chl a/b LHC</td>
<td>More (70-90) (phosphorylation-dependent)</td>
<td>Less (10-30) (phosphorylation-dependent)</td>
<td>Andersson and Anderson (1980); Kyle et al. (1983)</td>
</tr>
<tr>
<td>ATP synthase</td>
<td>0</td>
<td>100</td>
<td>Miller and Staehelin (1976)</td>
</tr>
</tbody>
</table>

(Taken from Staehelin, 1986)
ii) Light

Response to altered illumination leads to change in energy distribution between PS I and PS II (Bonaventura and Myers, 1969; Murata, 1969; Vernotte et al., 1975; Chow et al., 1981). Alteration in the LHC pigment bed in response to excess light trapping by PS I or PS II respectively is referred to as State I - State II transitions.

The biochemical mechanism regulating the spillover (state transition) of excitation energy has been demonstrated by Bennet (1977, 1979, 1980). It was shown that a membrane bound kinase and phosphatase catalyse the reversible phosphorylation of Chl a/b LHC proteins. The activation of this kinase is dependent on the oxidation-reduction state of the PQ pool (see Fig.1.5) (Horton and Black, 1980; Allen et al., 1981). Phosphorylation of the Chl a/b LHC protein preferentially directs the excitation energy to PS I and in the dephosphorylated 'state', the energy is preferentially directed to PS II (Bennett et al., 1980). The redox state of the electron transport chain thus regulates the turnover rate of the two reaction centres via Chl a/b LHC phosphorylation. The phosphorylation/dephosphorylation of Chl a/b LHC is uncoupler insensitive. The site(s) of phosphate incorporation is/are one or both threonine residues contained in the surface exposed Chl a/b LHC polypeptide segment which is necessary for grana formation (see Staehelin and Arntzen, 1983). The increased negative charge in the vicinity of PS II due to phosphorylation of Chl a/b LHC (Kyle and Arntzen, 1983) and the resulting conformational change of Chl a/b LHC is likely to cause destacking of the membranes and lateral migration of LHC (mobile form) from appressed to non-appressed regions (Staehelin and Arntzen, 1983). Circumstantial evidence suggests that the LHC might attach to PS I (Holzwarth, 1987). The mobile LHC and the structural arrangement of PS II-rich grana and PS I-rich stroma membranes allow a regulatory control of energy distribution via protein phosphorylation to ensure maximal efficiency of the two photosystems acting in series.

iii) Temperature

Higher temperatures than ambient induce inhibition of photosynthetic activities, mainly the photophosphorylation and PS II-mediated electron transport rate. The light harvesting chlorophylls become dissociated from the reaction centre and this results in loss in efficiency of excitation energy transfer.

Heat induced structural changes include (1) decrease in the amount of
Fig. 1.5 Schematic diagram of the feedback mechanism that controls the phosphorylation of the Chl a/b LHC particles and thereby the distribution of excitation energy between PS II and PS I. The redox potential of plastoquinone (PQ) pool serves as indicator of the turnover rate to the two photosystems. Accumulation of reduced PQ reversibly activates the kinase that phosphorylates the Chl a/b LHC.

(Taken from Staehelin, 1986)
stacked membrane regions, (2) decrease in size of EF particles, and (3) formation of phase separated aggregates of non-bilayer forming lipids (Armond et al., 1980; Gounaris et al., 1984).

Chilling induced injury in plants, on the other hand, is related with gel to liquid crystal phase transitions of membrane lipids.

3. Photosynthetic Light Reactions

a) The molecular orientation of pigments

The chlorophyll pigment is non-covalently bound to proteins (Thornber et al., 1979; Hiller and Goodchild, 1981; Kaplan and Arntzen, 1982). The binding of chlorophylls to proteins ensures (1) specific orientation and distance among pigments, which influence the probability of dipole-dipole coupling and (2) creates unique local chemical domains that determine the absorption properties of the pigment. These local domains create defined patterns of spectral species that ensure energy migration in a favourable way following an energetic gradient to pigments having the longest wavelength absorption properties (Govindjee and Govindjee, 1975). The absorption of a photon by a pigment molecule is followed by energy transfer in a random way throughout the antenna by resonance energy coupling of the dipole oscillations of interacting chromophores (Knox, 1977).

b) Excitation transfer

Upon absorption of a photon by chlorophyll molecule, an electron from the ground state is transferred to a higher energy state.

Excitation transfer among the antenna pigment molecules may take place by several mechanisms (see Knox, 1986) like occurrence of delocalized excitons and inductive resonance (Forster) transfer (Forster, 1965). The interactions are greatest among identical participating molecules, however, the interaction amongst Chl a and Chl b is also quite significant.

Other theories have also been proposed for excitation transfer (see Paillotin, et al., 1979; Pearlstein, 1982).

c) Energy trapping and charge separation

In chloroplasts, the excitation energy in light harvesting antenna is finally trapped in photosystem II reaction centre chlorophyll (P680) and in photosystem I reaction centre chlorophyll (P700). This leads to charge separation as
As a result of this primary electron transfer step, the charges (negative and positive) get spatially separated and get localized on different molecular species. The possibility of back reaction (charge recombination) after this primary charge separation is prevented by a very fast electron transfer (in less than 1 ns) from primary electron acceptor to secondary acceptor and reduction of oxidised primary donor (P680 or P700) by secondary donors.

The highly ordered molecular arrangement of electron transport components is essential in determining energy trapping and charge separation.

d) Probing of energy trapping by fluorescence yield and magnetic properties of reaction centres

Chlorophyll fluorescence provides an intrinsic probe for determining the energy transfer and the kinetics of charge separation in the photosynthetic apparatus. Improvement in the measuring techniques for recording fluorescence decays in the subnanosecond and picosecond time range (Breton and Gaecintov, 1980), phase-shift techniques (Moya and Garcia, 1983) and single photon timing techniques (Sauer and Brewington, 1978; Holzwarth et al., 1982; Haehnel et al., 1982) has now made it possible to measure the fast energy transfer processes. Synchronously pumped dye laser along with the single photon timing system can selectively excite specific pigment clusters.

It is now generally agreed that the major part of the short decay component of the Chl fluorescence (at 685 nm) arises from quenching of the Chl antenna by the open PS II centres (Haehnel et al., 1982; Nairn et al., 1982; Haehnel et al., 1983; Holzwarth et al., 1984), and the amount of long-lived fluorescence should reflect the percentage of closed centres. This component should be absent in fully open PS II. The origin of the long-lived component has been attributed to a delayed fluorescence induced by charge recombination in centres with reduced secondary acceptor Q (Klimov et al., 1978). This model has been widely accepted, however, it has been questioned (Haehnel et al., 1983) on the basis of the decrease in amplitude of the fast decay component upon
closing of PS II centres. Till recently three or four decay components of in vivo Chl fluorescence have been reported (life times in the range of 50-150 ps, 450-750 ps, 0.25-1.4 ns and 0.45-2.6 ns) but their origin is not yet fully agreed upon by various workers (see Holzwarth, 1986; Mathis, 1987; Hodges et al., 1987).

In the electron transfer steps of photosynthesis, unpaired electrons are located on molecules for a specific period of time. Radicals thus produced can be detected by ESR and related techniques. The interactions between two electrons of two radicals in a radical pair and formation of triplet states as a result of charge recombination have been studied by Optically Detected Magnetic Resonance (ODMR) (Clarke, 1982). Other techniques such as Electron Nuclear Double Resonance (ENDOR) (Norris et al., 1978, 1979; Lubitz et al., 1981; Lendzian et al., 1981; O'Malley and Babcock, 1984a; Lubitz et al., 1984), Fluorescence Detected Magnetic Resonance (FDMR) (Schaafsma, 1982; Hoff, 1982), Absorption Detected Magnetic Resonance (ADMR) (Blanken and Hoff, 1982, 1983) and Reaction Yield Detected Magnetic Resonance (RYDMR) have been applied to primary radical pair state in bacterial photosynthesis (Bowman et al., 1981; Norris et al., 1982; Wasielewski et al., 1983, 1984).

4. Energy Transduction
a) Role of photosystem II and photosystem I
i) Photosystem II complex

The photosystem II is the site of (1) primary event of charge separation associated with the reaction centre chlorophyll P680 and primary electron acceptor Qₐ, and (2) photooxidation of water. This involves electron transport from H₂O to PQ.

Several procedures have been used to isolate highly active oxygen evolving PS II preparation using different detergents like digitonin (Wessels et al., 1973; Satoh and Butler, 1978; Satoh, 1979), Triton X-100 (Berthold et al., 1981; Yamamoto et al., 1981, 1982; Klimov et al., 1982; Kuwabara and Murata, 1982, 1983; Nanba and Satoh, 1987) and β-octylglucoside (Green and Camm, 1984; Tang and Satoh, 1985; Ghanotakis and Yocum, 1986a,b). Inside out vesicles enriched in PS II and highly active in oxygen evolution have been prepared by means of phase partitioning of mechanically disrupted thylakoids (Åkerlund and Jansson, 1981; Åkerlund et al., 1982).

The PS II preparation is composed of several polypeptides, 47, 43, 34, 33, 32, 25-27, 24, 18 and 9 kD. Some polypeptides having low molecular weights,
7, 6.5, 5.5 and 5 kD have recently been reported (Ljungberg et al., 1986). (For details of functional role of polypeptides and their organisation in the membrane see section 6).

The reaction centre of PS II consists of chlorophyll a, P680, which is the primary donor, two pheophytin molecules (Pheo), a bound plastoquinone electron acceptor $Q_A$ and an electron donor $Z$. A second bound plastoquinone electron acceptor $Q_B$ is present in the 32 kD herbicide binding protein (Govindjee et al., 1985). A schematic representation of electron flow and its kinetics in photosystem II is shown in Fig.1.4.

ii) Photosystem I complex

The photosystem I is able to use light energy to separate a pair of charges and then stabilizes them (Kaplan and Arntzen, 1982). The primary electron acceptor (Chl a monomer) on getting photoreduced transfers the electron through non-heme Fe-S centre, Fd, Fp and quinone like intermediates to NADP$^+$. The photo-oxidized donor receives an electron from PS II via the cytochrome $b_6$-f complex and plastocyanin (PCy). Under some circumstances electron flow through PS I is cyclic in nature and is mediated by cytochrome $b_6$-f complex (Cramer and Crofts, 1982).

Several procedures have been used to isolate PS I particles giving various degrees of purity (Boardman and Anderson, 1964; Anderson and Boardman, 1966; Vernon et al., 1966; Wessels, 1966). Later procedures involved Triton X-100 and centrifugation (Vernon and Shaw, 1971), SDS and hydroxylapatite chromatography (Thornber, 1969; Dietrich and Thornber, 1971), Triton X-100 and hydroxylapatite chromatography (Shiozawa et al., 1974; Thornber et al., 1977), digitonin (Nelson and Racker, 1972), Triton X-100 and DEAE chromatography (Bengis and Nelson, 1975). The purified PS I reaction centre complexes were active in NADP$^+$ photoreduction when ascorbate served as an electron donor. Presence of Fd, ferrodoxin-NADP-reductase and PCy was essential for the reaction (Bengis and Nelson, 1975).

The PS I complex (Fig.1.6) is composed of six to seven different polypeptides which are designated as subunits I to VII in the order of decreasing molecular weights from 70 kD to 8 kD (Bengis and Nelson, 1975, 1977; Nelson and Notsani, 1977). Subunit I (70 kD) is denoted as the P700 reaction centre. It has been isolated in pure form (Bengis and Nelson, 1975) and is active in light-induced P700 oxidation and it also contains the primary electron acceptor,
Fig. 1.6 Schematic representation of the arrangement of polypeptides and electron transfer in PS I.

(Taken from Ort, 1986)
A\textsubscript{1,1} (Nelson and Notsani, 1977). Two copies of 70 kD polypeptide contain the reaction centre pigment P700 and also the light-harvesting antenna which is made up of 40 Chl a molecules and one \(\beta\)-carotene molecule (Bengis and Nelson, 1977).

The mutual orientation of pigments in photosystem I reaction centre has been studied by Junge et al. (1977a; Junge and Schaffernicht, 1979) by photo-selection with polarized light. The P700 pigment is probably situated in the internal side of the chloroplast membrane. The subunit I has been shown to be a transmembrane polypeptide by specific antibody studies (Bengis and Nelson, 1975, 1977; Nelson and Bengis, 1975). The subunit III (20 kD) functions on the oxidising side of PS I reaction centre either by providing the binding site for PCy or by inducing a proper conformation of subunit I (Bengis and Nelson, 1977; Haehnel et al., 1980). The other subunits IV (18 kD), V (16 kD) and VI (8 kD) (the three iron sulphur proteins) have been suggested to contain the secondary electron acceptors, A\textsubscript{1,2}(X), A\textsubscript{1,3}(B) and A\textsubscript{1,4}(A) (Bengis and Nelson, 1977; Okamura et al., 1982). The function of subunit II (25 kD) is not yet clearly understood.

The primary reactions of PS I have been shown in Fig.1.4. The exact nature of P700 as to whether it is chlorophyll a dimer or monomer is still not known (Rutherford and Heathcote, 1985). PS I photochemistry is distinguished from that of PS II and of purple bacteria by the presence of iron-sulphur type acceptors A\textsubscript{1,2}(X), A\textsubscript{1,3}(A) and A\textsubscript{1,4}(B). These are denoted as F\textsubscript{x}, F\textsubscript{B} and F\textsubscript{A} in the figures 1.4 and 1.6. They act as tertiary electron acceptors and can function at cryogenic temperatures. The redox centres of PS I reaction centre have been characterised by their mid-point potentials and their ESR spectra (see Okamura et al., 1982; Rutherford and Heathcote, 1985).

b) Mobile electron carriers and cytochrome b\textsubscript{6}-f complex

The photosystem II is spatially separated from photosystem I and the rapid transfer of electrons between stroma and grana regions is mediated by mobile electron carriers. The lateral movement of the electron carriers and their diffusion coefficient have been studied by several workers (see Whitmarsh, 1986).

The lipid soluble PQ accepts electrons from the bound-quinones acting on the acceptor side of PS II. This mobile PQ pool acts as a shuttle to transfer laterally electrons and protons along the lipid bilayer (Cramer and Crofts, 1982).
Fig. 1.7 Electron transfer in the cytochrome b$_6$f complex. Two sites of plastoquinone oxidation-reduction are involved, which are located on the outer (site C) and the inner (site Z) faces of the membrane.

(Taken from Joliot and Joliot, 1986)
The cytochrome b₆-f complex acts as the electron acceptor for PQH₂ (Fig.1.7). Evidence for two quinone binding sites has been reported (Jones and Whitmarsh, 1987). The redox activity of cytochrome b₆-f complex is coupled to the electron transfer to PS I via PCy (Mr = 10.5 kD). In spinach chloroplasts there are two molecules of PCy for each cyt b₆-f complex and PS I reaction centre (Graan and Ort, 1984). The oxidation-reduction of PCy takes place at the inner surface of the membrane. The diffusion coefficient of plastocyanin at room temperature has been determined as 10⁻⁸ cm² s⁻¹ (Selak and Whitmarsh, 1984).

The cytochrome b₆-f complex is a non-pigmented protein complex. It was first isolated and characterised from chloroplasts by Nelson and Neumann (1972). Ke et al. (1975) described its isolation using Triton X-100. This preparation contained a Cu-containing protein similar to PCy. A highly resolved cytochrome b₆-f complex was reported by Hauska et al. (1981) and Hurt and Hauska (1981). The preparation contained two molecules of cyt b₆ and two non-heme iron per cyt f. The preparation consisted of five polypeptides of 34, 33, 23, 20 and 17.5 kD, and was active in plastoquinol-plastocyanin oxidoreductase activity. The cytochrome f is present in the 34 kD polypeptide whereas the 23 kD protein might be the apoprotein for cytochrome b₆. The molecular weight of the Rieske iron-sulfur protein has been found to be 20 kD (Hauska, 1986). The Rieske iron-sulfur centre was first detected in mitochondrial bc₁ complex. It has a characteristic ESR signal (g = 1.89). The chloroplast cytochrome b₆-f complex shows a high degree of similarity in structure and function to the mitochondrial cytochrome bc₁ complex. The mutual arrangement of the subunits in all the b/c - Fe-S complexes is probably universal, with cytochrome b being largely embedded in the membrane as the most hydrophobic polypeptide. The Rieske Fe-S protein and cytochrome c₁ or f have hydrophilic parts (Hauska et al., 1984). By using an antibody against cytochrome f, it has been found that cytochrome f is located on the internal side of the thylakoid membrane. Allred and Staehelin (1986) have proposed the cyt b₆-f complex is spatially distributed throughout the thylakoid membrane and not partitioned in grana and stroma, like PS II and PS I.

c) The H⁺-ATPase complex and photophosphorylation

The electron transport in the above mentioned three protein complexes leads to the formation of an electrochemical potential difference of protons across the thylakoid membrane. The electro-chemical gradient of H⁺-ions is
composed of an electrical \((\Delta \psi)\) and a chemical component \((\Delta pH)\). The energy stored in the proton motive force is utilized to form ATP from ADP and inorganic phosphate by the \(H^+\)-ATPase complex. This enzyme known as \(CF_1-CF_0\) complex has a dual function; reversible catalysis of a chemical reaction and proton transport across the membrane (Strøtmann and Bickei-Sandkötter, 1984).

\[
3H^+_{\text{internal}} + ADP + P_i \xrightarrow{\Delta \psi} 3H^+_{\text{external}} + ATP + H_2O
\]

The \(CF_1-CF_0\) ATPase is a membrane bound protein complex and is highly analogous to \(F_1-F_0\) complex of mitochondrial inner membranes and bacterial membranes. The \(CF_1-CF_0\) complex is composed of two distinct structures, a catalytic sector that is hydrophilic in nature and a membrane sector that is hydrophobic in nature (Nelson, 1976, 1981). The catalytic sector (\(CF_1\)) catalyses the synthesis of ATP at the expense of energy stored in electrochemical gradient of protons. The membrane sector (\(CF_0\)) serves as a proton channel and provides the catalytic sector with a vectorial flux of protons across the membrane. The binding site of DCCD which is an inhibitor of ATP synthesis and proton transport is located in \(CF_0\).

\(CF_1\) has been isolated and purified by using several procedures (Avron, 1963; Binder et al., 1978; Farron, 1970; Lien and Racker, 1971; McEvoy and Lynn, 1973; Strøtmann et al., 1973; Younis, et al., 1977). The hydrophobic portion \((CF_0)\) of the ATPase complex was first isolated by Younis and Winget (1977). Pick and Racker (1979) have reported the isolation of the entire \(CF_1-CF_0\) complex.

The \(CF_1\) consists of five subunits named \(\alpha, \beta, \gamma, \delta\) and \(\epsilon\) in order of decreasing molecular weights 59, 56, 37, 17.5 and 13 kD respectively. A stoichiometry of \(\alpha_2\beta_2\gamma\delta\epsilon_2\) based on total molecular weight of \(CF_1\) as 325 kD had been proposed earlier by several workers (see Nelson, 1976). However, recently it has been reported that \(CF_1\) has the molecular weight of 390-400 kD with the five subunits \(\alpha, \beta, \gamma, \delta, \epsilon\) probably occurring in a ratio of 3:3:1:1:1 (see Merchant and Selman, 1985). The functions of the five subunits have been deduced from results of chemical modification, subunit specific antibodies and reconstitution studies. The \(\delta\) subunit is required for functional binding of \(CF_1\) to \(CF_0\). The active site is probably contained in the \(\beta\) (and \(\alpha\)) subunit(s). The \(CF_0\) consists of three polypeptides, designated as subunit I, II and III in order
of decreasing molecular weight (15, 12.5 and 8 kD respectively) (see Pick and Racker, 1979; Nelson et al., 1980). The 8 kD polypeptide is known to be the DCCD binding proteolipid (Nelson et al., 1977) which functions as a proton channel. Specific function for each individual subunit has been proposed and are summarised in Table 1.2 (Nelson, 1976, 1981).

5. Reconstituted Systems: Thylakoid Components Incorporated into Artificial Membranes

Purified CF$_1$-CF$_0$ complex reconstituted with lipids has been shown to give uncoupler sensitive ATP-P$_i$ exchange and acid-base driven ATP formation (Pick and Racker, 1979). Phenazine methosulfate dependent cyclic photophosphorylation system was reconstituted by co-incorporating photosystem I and coupling factor complex into liposomes (Hauska, 1980). Soybean phospholipids were used in these studies. Subsequently thylakoid diacyl lipids were found to be better substitutes. Sigrist-Nelson and Azzi (1980) found that galactolipids were required for the formation of a proton channel, sensitive to dicyclohexylcarbodiimide (DCCD).

LHC II incorporated into artificial membranes has revealed the essential role of LHC II in cation-induced thylakoid stacking (McDonnel and Staehelin, 1980; Mullet and Arntzen, 1980; Ryrie et al., 1980). Electron microscopic studies of model LHC II membranes show similar appression as in thylakoids; divalent cations are more effective than monovalent ones, and removal of a 2000 Dalton fragment from LHC II by trypsin treatment abolishes the cation effect.

Photosystem I has been reconstituted with Soybean phospholipids and is shown to exhibit an electrogenic proton translocation in light (Jaynes et al., 1975; Hauska, 1980; Orlich and Hauska, 1980). The plastoquinol-plastocyanin oxidoreductase activity of cytochrome b$_6$-f complex in model membranes have been shown to be stimulated by uncouplers and the other ionophores (Hauska et al., 1983).

Using low temperature fluorescence techniques Larkum and Anderson (1982) examined the spectral features of reconstituted vesicles containing LHC II and photosystem I or LHC II and the core complex of photosystem II. The results provide evidence for excitation transfer from 'free' LHC II units to either of the photosystems. Murphy et al. (1984) demonstrated that energy transfer can occur between 'free' LHCII and PS II already containing LHCII. This was shown by LHC II dependent enhancement of PS II activity in vesicles containing
Table 1.2 Proposed functions of the various polypeptides of coupling factor complex

<table>
<thead>
<tr>
<th>Subunit</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF₁</td>
<td></td>
</tr>
<tr>
<td>α</td>
<td>High affinity regulatory binding sites</td>
</tr>
<tr>
<td>β</td>
<td>Active site</td>
</tr>
<tr>
<td>γ</td>
<td>Energy transduction ?</td>
</tr>
<tr>
<td>δ</td>
<td>Binding of CF₁ to the membrane</td>
</tr>
<tr>
<td>ε</td>
<td>ATPase inhibitor</td>
</tr>
<tr>
<td>CF₀</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Binding of CF₁</td>
</tr>
<tr>
<td>II</td>
<td>Tightening hexamers of subunit III</td>
</tr>
<tr>
<td>III</td>
<td>Proton channel</td>
</tr>
</tbody>
</table>

(Taken from Nelson, 1982)
LHC II and the oxygen evolving PS II.

Attempts have also been made to reconstruct the complete electron transport chain from individual components (Lam and Malkin, 1982).

6. The Photosystem II

The photosystem II consists of several polypeptides: The probable function of the polypeptides is summarised in Table 1.3.

The reaction centre core of PS II was earlier proposed to be located on the 47 kD protein. In previous studies only the 47 and 43 kD proteins were found to carry Chl a. The site of the reaction centre was identified as 47 kD since this protein gave a fluorescence peak at 695 nm at 77 K (Naktani et al., 1984) which seemed to be characteristic of the reaction centre. The proposed model for the arrangement of the various polypeptides in the membrane is shown in Fig.1.8.

Recent developments, showing the remarkable similarities in functional and structural organisation of reaction centres of purple bacteria and PS II components from higher plants (Rutherford, 1985) propose that the 32 and 34 kD polypeptides (the D-1 and D-2) take part in P680 binding (Diesenhofer et al., 1985; Trebst and Depka, 1985; Trebst, 1986). The L- and M- subunits of the bacterial reaction centre show a high degree of amino acid sequence homology to the 32 (D-1) and 34 (D-2) PS II proteins of higher plants (Zurawski et al., 1982; Rochaix et al., 1984; Alt et al., 1984; Debus et al., 1985). The D-1 and D-2 proteins span the thylakoid membrane in 5-helices as their counterparts in bacterial chromatophores. From X-ray data of the crystallized reaction centre from *Rhodopseudomonas viridis* the exact positions of the prosthetic groups were deduced (Zurawski et al., 1982; Diesenhofer et al., 1984). The amino acid residues involved in binding of prosthetic groups are conserved in the 32 and 34 kD polypeptides in PS II of higher plants (Youvan et al., 1984; Rochaix et al., 1984; Trebst and Depka, 1985). Chl-binding polypeptides in the region of 30-34 kD have been reported (Irrgang et al., 1986; Nanba and Satoh, 1987). A reaction centre core complex consisting of the 34 kD, 32 kD and 9 kD proteins has been isolated and shown to be photochemically active (Satoh and Nanba, 1987; Nanba and Satoh, 1987; Okamura et al., 1987). These results suggest that the 32 and 34 kD proteins constitute the PS II reaction centre, whereas the 47 and 43 kD proteins could be antenna proteins. The 47 kD protein may also be involved in
Table 1.3 Proposed functions of the proteins of photosystem II

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PS II Core Complex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>47 kDa</td>
<td>Core antenna</td>
<td>Camm and Green (1983a, 1983b); Nakatani et al. (1984)</td>
</tr>
<tr>
<td>43 kDa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>34 kDa</td>
<td>Reaction centre</td>
<td>Satoh and Nanba (1987); Okamura et al. (1987)</td>
</tr>
<tr>
<td>32 kDa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 kDa</td>
<td>Cyt b-559</td>
<td>Herrmann et al. (1984)</td>
</tr>
<tr>
<td>4 kDa</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Extrinsic Proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>33 kDa</td>
<td>Mn stabilizer</td>
<td>Miyao and Murata (1984); Ono and Inoue (1984); Kuwabara et al. (1985); Miyao and Murata (1985)</td>
</tr>
<tr>
<td></td>
<td>Decrease in Cl⁻ requirement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acceleration of $S_3 \rightarrow S_0$</td>
<td>Miyao et al. (1987); Dismukes and Siderer (1980)</td>
</tr>
<tr>
<td>24 kDa</td>
<td>Ca²⁺ trap</td>
<td>Ghanotakis et al. (1984b); Andersson et al. (1984b); Miyao and Murata (1985)</td>
</tr>
<tr>
<td></td>
<td>Decrease in Cl⁻ requirement</td>
<td></td>
</tr>
<tr>
<td>18 kDa</td>
<td>Decrease in Cl⁻ requirement</td>
<td>Miyao and Murata (1985); Akabori et al. (1984)</td>
</tr>
<tr>
<td><strong>Hydrophobic Proteins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 kDa</td>
<td>Minor Chl-binding protein</td>
<td>Dunahay and Staehelin (1986)</td>
</tr>
<tr>
<td>22 kDa</td>
<td>Binding of 23-kDa protein</td>
<td></td>
</tr>
</tbody>
</table>

(Taken from Murata and Miyao, 1987)
Fig. 1.8 Schematic representation of the arrangement of polypeptides in the oxygen evolving PS II complex considering that the 47 kD polypeptide contains the primary reactants.

(Taken from Mathis, 1987)
binding of the electron donors to P680 and/or acting as a specific reaction centre antenna (see Mathis, 1987). The recent model for the PS II complex is shown in Fig.1.9.

a) The reaction centre complex

Light absorption in the antenna pigments generates an electronic excitation, which migrates from one molecule to the next and reaches the photochemically active chlorophyll with absorption maxima near 680 nm. Excitation of P680 results in the charge separation and formation of the primary radical pair P+I. The intermediate electron acceptor I has been identified as a pheophytin molecule (Klimov et al., 1977; Klimov and Krasnovskii, 1981; Klimov, 1984). Probably the electron is transferred to I via another intermediate Chl a (Fujita et al., 1978; Klimov et al., 1978, 1980a, b and c; Sauer and Brewington, 1978) analogous to bacterial reaction centres. From I the electron is transferred to QA, the primary electron acceptor. QA is a tightly bound plastoquinone molecule and is reduced to the unprotonated, anionic semiquinone form accompanied by electrochromic band shifts of pheophytin a molecule one of which is the 'C-550' (van Gorkom, 1974). Fig.1.10 represents a scheme for energy transfer in PS II reaction centre core. The ESR properties of QA are determined largely by its interaction with a nearby non-heme iron (Nugent et al., 1981). The g-value of QA Fe2+ signal is pH dependent (Rutherford and Zimmerman, 1984) and the presence of QA Fe2+ causes a splitting of the ESR signal of I (Klimov et al., 1980a). The QA is now known to correspond with Q400 (Petrouleas and Diner, 1984, 1986). It is characterized by a pH-dependent redox midpoint potential of 400 mV at pH 7.0 (with a change of -60 mV/pH unit), rapid photoreduction (within 1 μs) and very slow reoxidation rate. ESR signals in the high spin region due to interaction between QA and Fe atom has been recently reported (Itoh et al., 1986). The iron atom also interacts with the secondary acceptor quinone QB (Rutherford, 1987).

The secondary acceptor QB is bound to 32 kD protein, which is also identified as a herbicide binding protein. This polypeptide binds specific inhibitors of electron transport like DCMU that compete with QB in binding to 32 kD protein (Pfister et al., 1981). The 32 kD protein is coded in the chloroplast genome by a photogene D-1 which turns over rapidly in the light (Eaglesham and Ellis, 1974; Edelman and Reisfeld, 1978). The 34 kD protein (the D-2 protein) is also encoded by chloroplast genome. The amino acid sequence of the D-1
Fig. 1.9 A model for the oxygen evolving PS II complex, showing the involvement of 32 and 34 kD polypeptides with the reaction centre components. 

(Taken from Murata and Miyao, 1987)
Fig.1.10 Scheme for energy transfer and electron redistribution in PS II reaction-centres.

The times are reaction half-times

S  Singlet state
T  triplet state
ω  frequency of singlet-triplet transitions.

States including (Q) indicate the necessity of prereduced Q for triplet state formation.

The vertical distances between the energy levels of various redox states are approximate only.

(Taken from Ke, 1983)
and D-2 proteins have nearly identical hydrophobic/hydrophilic profiles (Rochaix et al., 1984) and strong homology is conserved between higher plants and bacteria. Mutations at three sites in the D-1 protein result in different patterns of herbicides resistance (Erickson and Rochaix, 1985). It has been proposed that the $Q_B$ binding protein is also the site of photoinhibition which inactivates PS II (Ohad et al., 1984; Kyle et al., 1984). The recovery from photoinhibition in light is controlled by the photogene (mentioned above). However, there are reports that the site(s) of photoinhibition may be the reaction centre, the $Q_A$ or elsewhere (Arntz and Trebst, 1986; Nedbal et al., 1986).

b) The oxygen evolving complex

Three membrane bound polypeptides having molecular weights of 33 kD, 23-24 kD and 16-18 kD have been shown to be part of the oxygen evolving complex (Åkerlund and Jansson, 1981; Yamamoto et al., 1981; Metz et al., 1981; Åkerlund et al., 1982; Kuwabara and Murata, 1982; Toyoshima and Fukutaka, 1982). These polypeptides have been localized on the inner surface of thylakoid membranes as established by the studies with inside-out vesicles (Åkerlund and Jansson, 1981; Åkerlund et al., 1982). Various treatments inhibit oxygen evolution by releasing or inactivating specific polypeptides and/or Mn (Table 1.4). All the three polypeptides are hydrophilic with polarity index equal to 48-49%, however, the existence of hydrophobic regions is possible. These proteins are non-pigmented and do not contain any metal ions. Some of the characteristics of these proteins are summarised in Table 1.5.

Rebinding studies and the ion-dependent association of these proteins with thylakoid membrane, suggest that the 16 kD (18 kD) protein is bound to the 23 kD (24 kD) and the 23 kD (24 kD) has a binding site on the 33 kD protein (Murata et al., 1983; Andersson et al., 1984a). A tentative model for the organisation of the oxygen evolving complex is shown in Fig.1.11.

Chloride has been shown to be a necessary cofactor in oxygen evolution (Critchley, 1985). The 24 and/or 18 kD proteins could be substituted by Cl$^-$ and/or Ca$^{2+}$ (Andersson et al., 1984b; Ghanotakis et al., 1984a; Miyao and Murata, 1984; Nakatani, 1984). Requirement for the 24 and 18 kD proteins in oxygen evolution is strongly dependent on the Cl$^-$ concentration in the medium (Andersson et al., 1984b; Imoaka et al., 1984). The addition of 23 kD (24 kD) protein to PS II preparation depleted of this protein lowered the Cl$^-$ requirement from
Table 1.4  Treatments releasing polypeptides from everted thylakoids concomitant with inhibition of oxygen evolution

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Inhibition of H₂O evolution</th>
<th>Release protein(kD)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M Tris-HCl (pH 9.3)</td>
<td>++</td>
<td>+ + + + + + +</td>
<td>Murata et al. (1983)</td>
</tr>
<tr>
<td>0.25 M NaCl (pH 7.4)</td>
<td>+</td>
<td>0 + + 0</td>
<td>Andersson et al. (1984a)</td>
</tr>
<tr>
<td>1 M NaCl (pH 6.5)</td>
<td>+</td>
<td>0 + + + + 0</td>
<td>Murata et al. (1983)</td>
</tr>
<tr>
<td>1 M NaCl/50 mM cholate</td>
<td>+ +</td>
<td>+ + + + +</td>
<td>Fukutaka et al. (1983)</td>
</tr>
<tr>
<td>1 M NaCl/2.5 M urea</td>
<td>+ +</td>
<td>+ + + + + + + +</td>
<td>Miyao and Murata (1983)</td>
</tr>
<tr>
<td>1 M CaCl₂ (pH 6.5)</td>
<td>+ +</td>
<td>+ + + + + + 0</td>
<td>Ono and Inoue (1984)</td>
</tr>
</tbody>
</table>

(Taken from Andersson et al., 1985)

Partial (+) or complete (++) release or inactivation.
Table 1.5 Summary of 18, 24 and 33 kD polypeptides

<table>
<thead>
<tr>
<th></th>
<th>33 kD</th>
<th>24 kD</th>
<th>18 kD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Molecular mass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS-PAGE*+</td>
<td>33</td>
<td>23-24</td>
<td>16-18</td>
</tr>
<tr>
<td>Gel filtration*</td>
<td>34</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td><strong>Isoelectric point</strong></td>
<td>5.2 †</td>
<td>6.5*; 7.3 †</td>
<td>9.5*; 8.5 †</td>
</tr>
<tr>
<td><strong>Absorption Maximum(λ_{max})</strong></td>
<td>276 nm*; 277 nm*†</td>
<td>277 nm*†</td>
<td>277 nm*†</td>
</tr>
<tr>
<td><strong>Extinction Coefficient, M^{-1} cm^{-1}(at λ_{max})</strong></td>
<td>20000*</td>
<td>26000†</td>
<td>13000†</td>
</tr>
<tr>
<td></td>
<td>18000†</td>
<td>24000†</td>
<td>12000†</td>
</tr>
<tr>
<td><strong>Polarity index</strong></td>
<td>49%*†</td>
<td>49%*†</td>
<td>52%*; 49%†</td>
</tr>
<tr>
<td>Extracted with 250 mM NaCl*†</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Extracted with 0.8 M Tris, pH 8.0*†</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Relation with Mn</strong></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Extracted with 2.5 M urea*</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Comments on amino acid composition</strong></td>
<td>Lacks histidine; is lysine-rich</td>
<td>Rich in aspartic acid, asparagine, lysine and glycine</td>
<td>Rich in leucine; poor in glycine; lacks methionine; contains lysine</td>
</tr>
<tr>
<td><strong>Cysteine residue</strong></td>
<td>3-4</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

(Taken from Govindjee et al., 1985)

*Kuwabara and Murata, 1983; Murata et al., 1983, 1984
†Jansson et al., 1983; Jansson, 1984
Fig.1.11 A tentative model for the organisation of the photosynthetic oxygen evolving system

- , hydrophobic binding
- , electrostatic binding

(Taken from Murata et al., 1983)
250 mM to 5 mM Cl\(^{-}\). It was thus concluded that the 23 kD (24 kD) protein was involved in the binding of Cl\(^{-}\). The 16 kD (18 kD) proteins was also demonstrated to be involved in Cl\(^{-}\) binding (Akabori et al., 1984), although high rates of O\(_2\) evolution can be maintained by 23 kD (24 kD) protein alone at Cl\(^{-}\) concentration above 3 mM. The 23 kD (24 kD) and/or 16 kD (18 kD) proteins also have a role in creating a high affinity Ca\(^{2+}\) binding site (Ghanotakis et al., 1984b). In salt-washed PS II particles, where the 24 (23) kD protein is absent, Ca\(^{2+}\) is released under illumination. In presence of 24 kD protein, Ca\(^{2+}\) is not released either in dark or in light. It has been concluded by Miyao and Murata (1986) that the main function of the 24 (23) kD protein is probably to retain Ca\(^{2+}\) in its functional site under illumination, which occurs by retention of optimum conformation of the Ca\(^{2+}\) binding protein.

The 34 kD protein was inferred to contain Mn atoms based on studies with mutant of *Scenedesmus obliquus* (Bishop, 1983). In chloroplasts a 34 kD protein containing Mn was reported by Abramowicz and Dismukes (1984). Two loosely bound Mn atoms may also be associated with the 33 kD extrinsic protein. The 33 kD protein was found to be essential to preserve Mn at the functional site. Mn probably binds at the interface between 33 kD and PS II core protein. The function of 33 kD protein as Mn stabiliser can be partially substituted by 150 mM Cl\(^{-}\). In absence of 33 kD protein, at [Cl\(^{-}\)] below 100 mM two of the four Mn atoms are released accompanied by inhibition of O\(_2\)-evolution (Murata and Miyao, 1987). Depletion of 33 kD protein also results in decrease in the rate of S\(_3\) to S\(_o\) transition from 5 ms to 15 ms (Miyao et al., 1987).

Mechanism of Oxygen Evolution

Joliot and Joliot (1968) discovered the oscillatory pattern of oxygen evolution under flashing light. This led to Kok's interpretation (Kok et al., 1970) of requirement of four oxidising equivalents in order to oxidise two water molecules. The different redox states of the donor side of PS II correspond to the redox states of the water-splitting system, denoted as S\(_n\) where n varies between 0 and 4 (see Bouges-Bocquet, 1980). In PS II particles Mn atoms are required for oxygen evolution and the change in their redox state is correlated to the S\(_n\) \(\rightarrow\) S\(_{n+1}\) transition (Dekker et al., 1984b). An intermediary donor, Z, has been characterised by its visible, ultra-violet and ESR spectra (Renger and Weiss, 1983; Dekker et al., 1984a, 1984c; Diner and de Vitry, 1984; O'Malley and Babcock, 1984b).

The stoichiometric details of H\(^{+}\)-liberation have not yet been completely solved. Some reports support a 0:1:1:2 stoichiometry (Junge et al., 1977b; Tieman et al., 1981). Recent kinetic studies favour a 1:0:1:2 sequence during S\(_o\) \(\rightarrow\) S\(_1\).
Several different models for the molecular mechanism of photosynthetic water oxidation have been proposed. The actual valence state of the manganese depends strongly on the nature of the overall coordination sphere. Investigation of synthetic manganese complexes shows that the redox potentials for the transitions $\text{Mn(II)} \rightarrow \text{Mn(III)} \rightarrow \text{Mn(IV)}$ can vary over a wide range for different ligand derivatives (Coleman et al., 1979; Okawa et al., 1982). The changes in the oxidation states of Mn during $O_2$ evolution in vivo have not yet been clearly understood. There is no unique pattern which could accommodate all the apparently conflicting data. The change in the oxidation state assignment to transition of $\text{S}_0 \rightarrow \text{S}_1 \rightarrow \text{S}_2 \rightarrow \text{S}_3 \rightarrow \text{S}_0$ transitions involving the $\text{fVIn(Ill)} \rightarrow \text{Mn(IV)}$ pattern $+1:+1:+1:-3$ was reported by Dekker et al. (1984b). This results in Mn oxidation state changes of $4\text{Mn}^{3+}; 3\text{Mn}^{3+} : \text{Mn}^{4+}; 2\text{Mn}^{3+} : 2\text{Mn}^{4+}; \text{Mn}^{3+} : 3\text{Mn}^{4+}; 4\text{Mn}^{3+}$. However, a pattern of $0:+1:0:-1$ seems to be more favourable (Velthuys, 1981; Lavergne, 1986). If only two Mn are involved; the $S$ states will involve $\text{S}_0 (\text{Mn}^{2+}/\text{Mn}^{3+}), \text{S}_1 (2\text{Mn}^{3+}), \text{S}_2 (\text{Mn}^{3+}/\text{Mn}^{4+})$ and $\text{S}_3 (\text{Mn}^{4+}/\text{Mn}^{4+})$ (Witt et al., 1987) (see Fig.1.12 and Table 1.6). If four Mn are involved; besides the two mentioned above, the third might be oxidised in $\text{S}_4$ and immediately re-reduced and the fourth might have only a stabilizing function (Witt et al., 1986, 1987).

A molecular mechanism of water oxidation in photosynthesis has been suggested by Kambara and Govindjee (1985). According to their model of the water oxidising complex, two Mn are enclosed in a hydrophobic cavity of intrinsic 34 kD and the other two are present on the hydrophilic surface of the 33 kD extrinsic protein. Each of the two Mn ions (in the hydrophobic environment) binds one redox active ligand (RAL) like quinone or aromatic amino acid residue. Electron transfer occurs from the reduced RAL to $Z^+$. Oxidation of $H_2O$ is carried out by these two Mn atoms and the protons are transferred to the other two Mn atoms (in the hydrophilic region) along the hydrogen bond. $Cl^-$ ion stabilizes the system by being indirectly coordinated to the Mn in the hydrophilic region as an outer sphere ligand. Sandusky and Yocum (1983) have suggested that $Cl^-$ may be the bridging ligand between Mn atoms.

The water oxidising system is connected to the reaction centre P680 by at least one redox component, $Z$ (or D). ESR spectra have shown $Z$ to be a
Fig. 1.12 Cleavage of water in PS II
(Taken from Witt et al., 1987)
Table 1.6 Stoichiometry and states of five events in the turnover of the water-splitting cycle

<table>
<thead>
<tr>
<th>Process</th>
<th>Sorbbed Quanta</th>
<th>Electron Extraction</th>
<th>Changes of Positive Surplus Charges</th>
<th>Intrinsic H⁺ Release</th>
<th>Possible States of Water</th>
<th>Possible States of Oxidizing Equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorption</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>OH⁻</td>
<td>Mn&lt;sup&gt;2⁺&lt;/sup&gt; Mn&lt;sup&gt;3⁺&lt;/sup&gt; Mn&lt;sup&gt;3⁺&lt;/sup&gt; Mn&lt;sup&gt;4⁺&lt;/sup&gt; Mn&lt;sup&gt;2⁺&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cycle</td>
<td>S&lt;sub&gt;0&lt;/sub&gt; → S&lt;sub&gt;1&lt;/sub&gt; → S&lt;sub&gt;2&lt;/sub&gt; → S&lt;sub&gt;3&lt;/sub&gt; → (S&lt;sub&gt;4&lt;/sub&gt;) → S&lt;sub&gt;0&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td>OH⁻</td>
<td>O⁻ O⁻ O⁻ -2 H₂O OH⁻</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mn&lt;sup&gt;2⁺&lt;/sup&gt; Mn&lt;sup&gt;3⁺&lt;/sup&gt; Mn&lt;sup&gt;3⁺&lt;/sup&gt; Mn&lt;sup&gt;4⁺&lt;/sup&gt; Mn&lt;sup&gt;2⁺&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mn&lt;sup&gt;3⁺&lt;/sup&gt; Mn&lt;sup&gt;3⁺&lt;/sup&gt; Mn&lt;sup&gt;4⁺&lt;/sup&gt; Mn&lt;sup&gt;4⁺&lt;/sup&gt; Mn&lt;sup&gt;3⁺&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Half-life times of the redox reactions of the oxidizing equivalents coupled with the S-state transitions

| Sorption                                    | 30 µs          | 110 µs              | 350 µs                              | 1.2 ms               |
| Cycle                                        | S<sub>0</sub> → S<sub>1</sub> → S<sub>2</sub> → S<sub>3</sub> → (S<sub>4</sub>) → S<sub>0</sub> |                      |                                      |                      |
|                                             | 50 µs          | 40 µs               | 80-120 µs                            | 1.2 ms               |
|                                             | 50 µs          | 100 µs              | 220 µs                              | 1.2 ms               |

(Taken from Witt et al., 1987)
special plastoquinol (PQH$_2$) that functions as a one electron carrier with PQH$_2^+$ as a cation radical (Blankenship et al., 1975; Ghanotakis, 1983). This is supported by difference absorption spectra of Z$^{Ox}$/Z couple (Dekker et al., 1984a, 1984c; Diner and deVitry, 1984; Weiss and Renger, 1984). It has been proposed that there are two donors Z$_1$ and Z$_2$ which could function in a parallel pathway as shown below (Bouges-Bocquet, 1980).

\[
\begin{align*}
H_2O & \rightarrow OEC \quad \left[ \begin{array}{c}
S_0, S_1 \text{ fast} \\
S_2, S_3 \text{ slow}
\end{array} \right] \\
& \rightarrow Z_1 \rightarrow P680 \\
& \rightarrow Z_2
\end{align*}
\]

Based on kinetic studies, presence of two donors (Z$_1$ and Z$_2$) have been suggested (Brettel et al., 1984a, 1984b). Z$_1$ gives rise to ESR signal $\Pi_{\Pi}$. A new ESR signal has been discovered at $g = 4.1$ (Casey and Sauer, 1984; Zimmerman and Rutherford, 1984). This ESR signal could arise from the intermediate donor Z$_2$.

The electron flow from Z to P680$^+$ occurs in 20 ns in the presence of $S_0$, $S_1$ states and 400 ns when the $S_2$, $S_3$ states are present (Brettel et al., 1984b).

The $S_2$ and $S_3$ states behave differently than the $S_0$, $S_1$ (see Joliot and Kok, 1975; Vermaas et al., 1984). They are sensitive to different substances such as ADRY reagents (agents which accelerate the deactivation reactions of system Y) (Renger et al., 1973).

c) The cytochrome b-559

Experimental evidence indicates that cyt b-559 is closely associated with PS II and may have a role in oxygen evolution. Mutants that lack cyt b-559 also lack PS II activity (Maroc and Garnier, 1981). Bishop (1984) has suggested that cyt b-559 may function as an intermediate between the OEC and Z. The release and rebinding of the 23 kD protein reversibly affects the redox potential of cyt b-559 (Larsson et al., 1984). The restoration of the high potential form (by incorporation of PS II in liposomes) coincides with increased rates of $O_2$ evolution (Matsuda and Butler, 1983). A hypothesis has been suggested in which the cyt b-559 functions on both the reducing as well as the oxidising sides of PS II (Butler and Matsuda, 1983). The energy difference between cyt b-559 (HP) ($E_{m,7} \approx 380$ mV) and cyt b-559 (LP) ($E_{m,7} \approx 80$ mV) might have a role in the
splitting of water by PS II. Cyt b-559 is found in the proximity of the D-1 and D-2 proteins (Nanba and Satoh, 1987) however the function of cyt b-559 in PS II is not established.

d) Heterogeneity in photosystem II

A heterogeneity in the rate of photoreduction of Q in higher plant chloroplasts was shown by Melis and Homann (1975) and attributed to the existence of two types of PS II centres, called $\alpha$ and $\beta$. PS II $\alpha$ and PS II $\beta$ have been shown to differ in their light harvesting Chl antenna size (Melis and Duysens, 1979; Anderson and Melis, 1983) and in their location. PS II $\alpha$ is found in granal membrane and PS II $\beta$ is located in stroma-exposed membrane regions together with PS I (Melis and Thielen, 1980; Anderson and Melis, 1983). PS II $\beta$ has a smaller LHC than PS II $\alpha$ (Thielen and van Gorkom, 1981a) and the absorption maxima of Chl is shifted towards the red region as compared to that of chlorophylls of PS II $\alpha$ (Thielen et al., 1981). PS II $\alpha$ is associated with the two-electron gate Q whereas PS II $\beta$ is not (Thielen and van Gorkom, 1981b). The primary stable electron acceptors, termed $Q_\alpha$ and $Q_\beta$ for PS II $\alpha$ and PS II $\beta$ respectively, have been found to have different midpoint redox potentials (Horton, 1981). PS II $\beta$ can be preferentially altered by high herbicide concentrations (Horvath et al., 1984). The two types of centres have different fluorescence emission characteristics (Brearley and Horton, 1984) and only the fluorescence emission of PS II $\alpha$ is affected by Mg$^{2+}$ levels (Melis and Ow, 1982). It has also been suggested that during transition from state 1 to state 2 the phosphorylated LHC preferentially attaches itself to the PS II $\beta$ centres (Holzwarth, 1987).

In contrast to the above, it has been suggested that the biphasic nature of the fluorescence rise may simply be due to different degrees of PS II connectivity and not a result of differences in antenna size or localization in stroma or grana regions (Bowes and Horton, 1982). The biphasicity of fluorescence rise has also been explained as (1) degree of chloroplast integrity linked to alterations in DCMU binding (Schreiber and Pfister, 1982; Hodges and Barber, 1984), (2) a consequence of different degrees of PS II/LHC interaction (Percival et al., 1984). It has also been suggested that the fluorescence rise is probably far more complex than just two phases (Hodges and Barber, 1986). Their results do not seem to be consistent with the concept of two spectrally and functionally distinct PS II. It has been proposed by Hodges and Barber (1986) that different degrees of DCMU inhibition of PS II particles may be sufficiently explained on the basis
of one type of PS II centre without resorting to the concept of heterogeneity.

e) Stoichiometry of various components

Quantitation of photosystem II in several plant chloroplasts by light-induced absorbance changes $\Delta A_{320}$ yielded chlorophyll/PS II ratios mostly in the range of 300-400 and reaction centre ratios RC II/RC I of 1.4-2.0 (Melis and Anderson, 1983; Melis, 1984; Glick et al., 1985). Using flash induced H$^+$ release from H$_2$O oxidation in presence of artificial electron acceptors, a value of 630 for Chl/PS II was obtained (Whitmarsh and Ort, 1984). This discrepancy has been suggested to be due to presence of two populations of PS II, active and inactive (Chylla et al., 1987).

In isolated photosystem II particles prepared from spinach chloroplasts using Triton X-100, the ratio of Chl/RC is around 220 (Murata et al., 1984). Stoichiometry of the various components Mn/Cyt b-559/Z/P680/Ph/QA/herbicide binding protein in isolated PS II has been estimated as 4:2:1:1:1:1 respectively (Berthold et al., 1981; Yocum et al., 1981; Lam et al., 1983; Vermaas and Arntzen, 1983; Jursinic and Stemler, 1983; Babcock et al., 1983; Ghanotakis et al., 1984c; Murata et al., 1984; Eckert et al., 1984; Golbeck and Warden, 1985).

7. Aim of the Present Study

The aim of the present study was to investigate the structural and functional relationship between the various components of isolated photosystem II membrane fragments from spinach. The following studies were carried out:

a) The effect of proteolytic digestion on normal and salt-washed PS II particles with trypsin/Chl = 0.0001 to 1. We have attempted to correlate the structural modifications with the functional properties after trypsin treatment.

b) The role of cyt b-559 in photosystem II particles and to analyse the functions of the different potential forms of cyt b-559 in the electron transport process. We studied the kinetics of cyt b-559 photooxidation and reduction in isolated PS II preparation suspended in buffer and in PS II particles incorporated into liposomes in the presence of an ADRY reagent N,N,N',N'-tetramethyl-p-phenylenediamine and electron transport inhibitor atrazine.

c) Study of the $\alpha$ and $\beta$ kinetics of fluorescence induction curves in isolated PS II particles in presence of electron transport perturbants. The effect of pH on various functional parameters of PS II $\alpha$ and PS II $\beta$ and the study whether
α and β-kinetics originate from structurally distinct PS IIs or they are interconvertible.

d) Investigation of the fluorescence polarization ratios after treatments which affect the membrane structure and organisation, namely trypsin treatment and salt-washing. To correlate the structural reorganisation with the function of the photosystem II particles, mainly the effect on the primary charge separation, the effect of (i) the herbicide, atrazine, which is effective on the acceptor side and (ii) TMPD, which acts on the donor side of PS II similar to reagents which accelerate the deactivation rate of system Y (ADRY reagents) on the fluorescence polarization ratio of Chl a and DPH in PS II particles was studied. These are described in chapters 2 to 6.

7. References


Chapter 2

ISOLATION AND CHARACTERISATION OF PHOTOSYSTEM II FROM SPINACH

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