Chapter 5

EFFECT OF HEAT AND HIGH IRRADIANCE ON PHOTOSYSTEM II PARTICLES RECONSTITUTED INTO ARTIFICIAL MEMBRANES

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5.1 Introduction

Photosynthetic electron transport in thylakoid membranes is achieved through an adequate spatial organization of chlorophyll-protein complexes linked together by protein and non-protein redox components (Staehelin and Arntzen 1983). All these components are distributed within a lipid environment which may not be considered as a simple homogeneous bilayer, but it is characterized by a transverse as well as lateral heterogeneity (Rawyler and Siegenthaler 1981, 1985, Giroud and Siegenthaler 1984, Gounaris et al. 1983, Henry et al. 1983, Chapman et al. 1984). Alterations in the lipid membrane environment may alter the conformation and orientation of proteins which may eventually affect functions of the photosynthetic apparatus.

The functional role of lipids in a given membrane is not clearly understood because lipids unlike most of the proteins have no recognized catalytic properties. However, it is known that depletion of certain lipids of thylakoid membranes by lipases result into a reduction of photosynthetic activities (Siegenthaler and Rawyler 1986, Siegenthaler et al. 1987). Although the studies on lipid hydrolysis have provided valuable information on the role of lipids in thylakoid membrane function (see Siegenthaler and
Rawyler 1986 for review), but a drawback of this technique is that the hydrolysis products resulting from the enzymatic reactions may, by themselves, interact with the photosynthetic components of the membrane and perturb it. There are evidences that exogenous (Siegenthaler 1973, 1974, Siegenthaler and Rawyler 1977) and endogenous (Siegenthaler 1974, Siegenthaler and Rawyler 1972, Siegenthaler and Depery 1977) free fatty acids and lysophospholipids (Hoshina and Nishida 1975) can considerably impair the structure and function of chloroplasts. Siegenthaler et al. (1987) have proved that the part of the total inhibition of photosystem II activity induced by phospholipase A₂ can be restored by removal of hydrolysis products by bovine serum albumin. However, this partial restoration of activity was only possible as long as the phospholipid depletion did not exceed a certain limit on 40 min. of incubation with phospholipases. On the basis of these results, Siegenthaler et al. (1987) have hypothesized that there are three functional lipid populations for both phosphatidylcholine and phosphatidylglycerol. The first one corresponding to about 40 percent of each lipid class can be removed without affecting greatly the non-cyclic electron flow. It is likely that most of these lipids belong to the outer monolayer. The second population of phospholipids (from 40 to 90 percent of phosphatidylcholine and from 40 to 80 percent for
phosphatidylglycerol) is of utmost importance to sustaining the activity. Depletion of this population of lipids results into drastic inhibition of electron transport activity. The third population of phospholipids which is present even after complete inhibition of electron transport activity does not appear to support non-cyclic electron flow and belongs entirely to the inner monolayer of the thylakoid bilayer.

Reconstitution of functional thylakoid complexes has been used by a number of workers to study the role of lipids in the energy transduction in chloroplasts (Murphy 1986a, Murphy 1986b, Ryrie 1986). Murphy et al. (1989) studied the efficacy of various acyl lipids in the restoration of energy transfer between solubilized chlorophyll-protein complexes and suggested that impure phosphatidylcholine, which is present in the thylakoid membrane in very minute quantities, was the best reconstituting agent. Other purified chloroplast lipids were relatively poor reconstituting agents. There was also a pronounced selectivity of thylakoid protein for different lipids. We have used phosphatidylcholine for reconstitution of isolated PSII particles. Phosphatidylcholine with fatty acyl residues of varying chain lengths from 14 carbon to 18 carbon were used in this study to investigate the role of hydrophobic core of
the lipid bilayer in the reconstitution of PSII activity into model membranes.

5.2 Materials and Methods

5.2.1 Isolation of photosystem II particles

Photosystem II particles were isolated from 8-10 days old wheat leaves using the procedure of Berthold et al. (1981) with the modification by Ghanotakis et al. (1984). Freshly prepared thylakoids were suspended in 50 mM MES-NaOH (pH 6.0), 15 mM NaCl, 5 mM MgCl₂ and 1 mM sodium ascorbate and Triton X-100, from a 25% (w/v) stock, was added dropwise to yield a detergent to chlorophyll ratio of 25:1. After 30 min. of stirring in dark on ice, the suspension was centrifuged at 4000xg for 4 min. to remove the starch. The supernatant was again centrifuged at 40,000xg for 30 min. and the pellet was resuspended in 50 mM MES-NaOH (pH 6.0), 15 mM NaCl, 5 mM MgCl₂ and the chlorophyll concentration was determined according to Arnon (1949). A second detergent treatment was performed by adding Triton X-100 to give 5 mg detergent/mg Chl. After incubation in dark for 5 min. the suspension was again centrifuged at 40,000xg for 30 min. The resulting pellet was resuspended in 400 mM sucrose, 50 mM MES-NaOH (pH 6.0), 15 mM NaCl and 5 mM MgCl₂ and was used as PSII particles for all the experiments.
5.2.2 Reconstitution of photosystem II particles into phospholipid vesicles

Diacyl phosphatidylcholines with different fatty acyl chain lengths dimyristoyl phosphatidylcholine (DMPC), dipalmitoyl phosphatidylcholine (DPPC) and distearoyl phosphatidylcholine (DSPC) (obtained from Sigma Chemical Co., USA) were used for preparation of multilamellar liposomes for reconstitution of isolated PSII particles. Weighed amounts of lipids dissolved in chloroform:methanol (2:1 v/v) were taken in round bottom flasks and were dried under nitrogen in a rotary evaporator to make a film of lipid on the wall of the flask. The lipid was then dispersed in buffer with PSII particles suspended in it at 10 μg Chl/ml. The suspension was vortexed vigorously for 15 min. to form the multilamellar liposomes with the PSII membrane fragments embedded within the lipid bilayers. The ratio of lipid:Chl in the reconstituted vesicles was always maintained constant at 20:1 (w/w).

5.2.3 Heat treatment of photosystem II particles

PSII particles either in buffer or reconstituted into phosphatidylcholine vesicles were heat treated in a temperature controlled water bath at desired temperatures for various periods of time as described in Chapter 3. Heat
treatment was terminated by bringing them back to ice. All the heat treatments for studying the effect of high temperature alone were given in the dark.

5.2.4 Photoinhibition of isolated photosystem II particles

PSII particles at 10 μg Chl per ml were kept in a jacketed glass container. The temperature of the samples was maintained constant at 25°C by circulating water from a temperature controlled water bath. Photoinhibitory light was provided by a slide projector (Parkeo automat S-250, Germany) which illuminated the sample chamber at 2250 W/m². The intensity of photoinhibitory light was varied by using a number of neutral density filters. Light treatment to the PSII particles was given for different periods of time depending on the experimental needs.

5.2.5 Interaction of heat stress and photoinhibition in isolated photosystem II particles

The effect of combination of heat stress and photoinhibition on isolated PSII particles was studied by sequentially subjecting them to various levels of the two stresses as described earlier. The order of the stresses was also changed to examine the changes in the extent of damage to the PSII particles.
Fig. 5.2 Photosynthetic electron transport rates (H₂O to DCIP) of isolated photosystem II particles in buffer (○) or in multilamellar vesicles of DMPC (●), DPPC (△) or DSPC (▲). PSI-I particles were exposed to different light intensities for 1 min. each before measuring the rates.
Fig. 5.3 Photosynthetic electron transport rates (DPC to DCIP) of isolated photosystem II particles in buffer (O) or in multilamellar vesicles of DMPC (●), DPPC (∆) or DSPC (▲). PSII particles were exposed to different light intensities for 1 min. each before measuring the rates.
activity by addition of DPC in the assay mixture increased to 4, 8 and 10 percent when photoinhibition treatment was given to PSII particles reconstituted into DMPC, DPPC and DSPC vesicles respectively. These results indicate that the lipid bilayer extends a protection to PSII particles against photoinhibition damage and phosphatidylcholines with longer fatty acyl residues provide better stability to the PSII complex.

5.3.1.2 High temperature stress

The rate of DCIP photoreduction in Triton X-100 solubilized thylakoid membrane fragments in buffer as well as in multilamellar vesicles of phosphatidylcholine was observed to decrease with increase in the temperature of heat treatment (Fig. 5.4). The reduction in electron transport activity was slow up to 30°C beyond which it decreased sharply which indicates that isolated wheat PSII particles are comparatively stable up to 30°C. High temperature induced reduction in electron transport activity of PSII particles is attributed to the damage to PSII complex. Nash et al. (1985) reported a similar decrease in oxygen evolution activity of spinach PSII particles on heat treatment. Thompson et al. (1986) have suggested that heat induced inactivation of oxygen evolving complex occurs due to
release of two Mn atoms from the oxygen evolving complex. Release of Mn has also been suggested to cause a decrease in the stability of D⁺ and reduction potential of cyt b₅₅₉ (Cramer et al. 1981). A number of proteins including 33, 23 (Volger and Santarius 1981), 43, 28 and 22 kDa (Thompson et al. 1989) polypeptides have also been reported to be released from thylakoid membranes and PSII particles after high temperature treatment. However, the proportion of protein release is always smaller than that of the activity loss. Thus it seems likely that both the release of functional manganese and polypeptides of oxygen evolving complex are caused by heat stress which eventually lead to the loss of electron transport activity.

It is apparent from the results that the protection of PSII particles from high temperature damage was provided by lipid bilayers in case of heat treatment of PSII particles reconstituted in phosphatidylcholine liposomes. The extent of protection against heat stress also varied with phosphatidylcholines with different fatty acyl residues. Distearoyl phosphatidylcholine (DSPC) with 18 carbon saturated fatty acyl residues extended the maximal protection against high temperature damage to the PSII complex. Fig. 5.5 shows the changes in electron transport activity of PSII particles in buffer and in phosphatidylcholine liposomes as a function of temperature of heat
Fig. 5.4 Electron transport activity (H₂O to DCIP) of isolated PSII particles after heat treatment at different temperatures for 1 min. in dark. ○: in buffer, ●: in DMPC vesicles, △: in DPPC vesicles, ▲: in DSPC vesicles.
Fig. 5.5 Electron transport activity (DPC to DCIP) of isolated PSII particles after heat treatment at different temperatures for 1 min. in dark. ○: in buffer, ●: in DMPC vesicles, △: in DPPC vesicles, ▲: in DSPC vesicles.
treatment in presence of DPC. Although the pattern of heat inactivation was same as in the case of electron transport from $H_2O$ to DCIP, but it is apparent from the diagram that electron transport from DPC to DCIP was less perturbed on heat treatment as compared to the electron transport chain from $H_2O$ to DCIP. Partial restoration in the electron transport activity of PSII particles in presence of exogenously added DPC suggests that part of the PSII electron transport chain prior to the site of electron donation by DPC is one of the targets of high temperature stress. These results are also in agreement with previous reports suggesting a high temperature induced damage to oxygen evolving complex of higher plant PSII complex (Katoh and San Pietro 1967, Cramer et al. 1981, Nash et al. 1985).

5.3.2 In vivo chlorophyll fluorescence

5.3.2.1 Photoinhibition

The characteristics of in vivo chlorophyll fluorescence induction were used as the second parameter to study the role of lipid bilayer in the functioning of PSII complex under environmental stress. There was a loss of variable chlorophyll fluorescence when wheat PSII particles were subjected to photoinhibitory treatment. The yield of variable chlorophyll fluorescence was reduced on photo-
inhibition of PSII particles reconstituted into phosphatidylcholine liposomes. The decrease in $F_v$ was, however, less in case of photoinhibition of PSII particles reconstituted into phosphatidylcholine liposomes (Fig. 5.6). This decrease in $F_v$ subsequently caused a reduction in the $F_v/F_m$ ratio of PSII particles in buffer as well as in phosphatidylcholine liposomes with different fatty acyl residues (Fig. 5.7). The ratio of variable to maximal chlorophyll fluorescence has been shown to be directly related to the maximal quantum yield of PSII photochemistry (Kitajima and Butler 1975) and photon yield of oxygen evolution (Adams III et al. 1990). The reduction of $F_v/F_m$ ratio of PSII particles on photoinhibition might reflect a damage to PSII particles which may be caused by degradation of D1 protein (Kyle et al. 1984). The extent of inhibition decreased with the increase in the number of carbon atoms from 14 in dimyristoyl phosphatidylcholine to 18 in distearoyl phosphatidylcholine liposomes (Fig. 5.6 and 5.7).

5.3.2.2 High temperature stress

A decrease in the variable fluorescence, $F_v$, was observed with increase in the temperature of heat treatment of PSII particles either in buffer or in phosphatidylcholine liposomes. In buffer, the variable fluorescence decreased by
Fig. 5.6 Variable chlorophyll fluorescence of isolated photosystem II particles in buffer and lipid vesicles. PSII particles were exposed to different light intensities for 1 min. each before measuring the rates. O: in buffer, •: in DMPC vesicles, Δ: in DPPC vesicles, ▲: in DSPC vesicles.
Fig. 5.7 $F_v/F_m$ ratio of isolated photosystem II particles in buffer and lipid vesicles. PSII particles were exposed to different light intensities for 1 min. each before measuring the rates. $\circ$: in buffer, $\bullet$: in DMPC vesicles, $\triangle$: in DPPC vesicles, $\blacktriangle$: in DSPC vesicles.
35 percent on heat treatment of PSII particles at 45°C, whereas the same treatment of PSII particles in DMPC, DPPC and DSPC liposomes resulted into a decrease of 22, 16 and 10 percent respectively (Fig. 5.8). The reduction in the efficiency of primary photochemistry of PSII complex was also reflected by decrease in the $F_v/F_m$ ratio of PSII particles either stressed in buffer or in phosphatidylcholine liposomes (Fig. 5.9).

It has been suggested that if photosynthetic electron transport is inhibited by any accompanying stress, the reaction center is unable to dispose the absorbed excitation energy, nor can it accept excitation energy from their harvesting pigment-protein complexes. The probability of transfer of electrons to oxygen present in the vicinity of PSII centers thus increases manifolds which may result in an increased generation of singlet oxygen which could damage the PSII components (Takahama and Nishimura 1975, Elstner 1982, Halliwell and Gutteridge 1989, Richter et al. 1990). This hypothesis was further substantiated by our observations that the reduction in variable chlorophyll fluorescence, $F_v/F_m$ ratio and decrease in electron transport activity was more when PSII particles were subjected to photoinhibition and heat stress simultaneously.
Fig. 5.8 Variable chlorophyll fluorescence ($F_v$) of isolated PSII particles after heat treatment at different temperatures for 1 min. in dark. ○: in buffer, ●: in DMPC vesicles, △: in DPPC vesicles, ▲: in DSPC vesicles.
Fig. 5.9 $F_v/F_m$ ratio of isolated PSII particles after heat treatment at different temperatures for 1 min. in dark. O: in buffer, •: in DMPC vesicles, △: in DPPC vesicles, ▲: in DSPC vesicles.
5.3.3 Interaction of high temperature and light stress

Heat and high light stress have been shown to interact at the level of thylakoid membranes (Al-Khatib and Paulsen 1989, Sayed et al. 1989a, 1989b). The interaction of high temperature and photoinhibition in PSII particles was studied by simultaneous imposition of the two stresses on PSII particles either in buffer or in phosphatidylcholine liposomes differing in their fatty acyl composition. The results show that at all the three temperatures (40, 45 and 50°C) and light intensities (590, 1200 and 1750 W/m²), the reduction in electron transport activity was less when DPC was present during the measurements (Table 5.1). These results suggest that donor side of PSII is one of the targets of heat stress and photoinhibition. The results are in agreement with the previous reports which suggest that oxygen evolution is one of the most heat sensitive processes of photosynthesis (Berry and Bjorkman 1980, Nash et al. 1985). An exacerbation of thermal injury by high light intensity was also observed in our study.

It is apparent from the results that the damage to PSII particles due to high temperature and photoinhibition is less when the PSII particles are reconstituted into phosphatidylcholine liposomes. The mechanism by which the phospholipid bilayer protects the PSII complex from high
Table 5.1

Effects of interaction of high temperature and light stress on photosystem II particles either in buffer and in multilamellar vesicles of phosphatidylcholines differing in fatty acyl chain length. The values of $F_v/F_m$ are shown up to three significant decimal points.

<table>
<thead>
<tr>
<th>PFD $W/m^2$</th>
<th>BUFFER</th>
<th>DMPC VESICLES</th>
<th>DPPC VESICLES</th>
<th>DSPC VESICLES</th>
</tr>
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<tr>
<td></td>
<td>$H_2O$</td>
<td>$DCIP$</td>
<td>$F_v$</td>
<td>$F_v/F_m$</td>
</tr>
<tr>
<td></td>
<td>to</td>
<td>to</td>
<td>to</td>
<td>to</td>
</tr>
<tr>
<td></td>
<td>% control</td>
<td>% control</td>
<td>% control</td>
<td>% control</td>
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<tr>
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<td></td>
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<td></td>
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<td>590</td>
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<td>14</td>
<td>62</td>
<td>0.827</td>
</tr>
<tr>
<td>1200</td>
<td>6</td>
<td>11</td>
<td>48</td>
<td>0.774</td>
</tr>
<tr>
<td>1750</td>
<td>3</td>
<td>9</td>
<td>48</td>
<td>0.774</td>
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<tr>
<td>50°C</td>
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<td></td>
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<td></td>
</tr>
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</tr>
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</table>

R.U. = Relative units
temperature and photoinhibition damage is not clear but it seems likely that the lipid bilayer extends stability to PSII against these environmental stresses.

5.3.4 Role of lipid bilayer in the functioning of PSII complex under stress conditions

PSII complex of higher plants contain a number of intrinsic membrane proteins (see Barber 1987 for review). The PSII complex reconstituted into liposomes may be considered as a pigment-protein complex traversing the lipid bilayer of the liposome. The possibility of interaction of PSII components with the lipid bilayer can not be ruled out because the reaction center proteins such as D1 and D2 have distinct hydrophobic domains which are expected to be buried within the core of lipid bilayer. For simple bilayer system of one lipid, the activity of reconstituted PSII complex is likely to be a complex function of number of factors which may include bilayer surface potential, bilayer thickness, bilayer lipid backbone and bilayer lipid acyl chain order. Phosphatidylcholines used in this study contained saturated fatty acyl residues varying in chain length from 14 carbon to 18 carbon.

A passive sugar transport protein from human erythrocyte with 55 kDa molecular mass having 12 membrane-
spanning hydrophobic and three hydrophilic domains (Mueckler et al. 1985) has been used as a model system for study of functioning of membrane proteins in different lipid environments (Carruthers and Melchior 1984a, 1984b, Connolly et al. 1985). It was observed that phospholipids with longer fatty acyl chains supported higher transport activities (Carruthers and Melchior 1984, Tefft et al. 1985). The results of our experiments also show that lipids with longer fatty acyl chain extend a functional stability to PSII complex against heat stress and photoinhibition. Acyl chain order (proximity of packing within the bilayer) and bilayer thickness has been demonstrated to increase with acyl chain length (Stroch et al. 1983) which may account for the stabilizing effects of phosphatidylcholines. Membrane fluidity, which is generally considered vital, seems to have limited implications in the functioning of a number of intrinsic membrane proteins and it has been suggested that factors such as fatty acyl chain length of bilayer lipids and saturation/unsaturation can override the effects of physical state (fluidity) of bilayer (Carruthers and Melchior 1986).