Bioelectrochemistry 73 (2008) 43–48

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Effect of aggregation state, temperature and phospholipids on photobleaching of photosynthetic pigments in spinach Photosystem II core complexes

A. Ventrella [a], L. Catucci [a,b,*], A. Agostiano [a,b]

[a] Dipartimento di Chimica, Universita' di Bari, Via Orabona 4, 70126 Bari, Italy
[b] IPCF — CNR, sezione di Bari, Via Orabona 4, 70126 Bari, Italy

ARTICLE INFO

Article history:
Received 21 May 2007
Received in revised form 14 March 2008
Accepted 7 April 2008
Available online 12 April 2008

Keywords:
Photobleaching
Photosystem II
Phosphatidylglycerol
Cardiolipin
Aggregation state

ABSTRACT

Photosystem II (PSII) complex activity is known to decrease under strong white light illumination, and this photoinhibition phenomenon is connected to the photobleaching of the PSII photosynthetic pigments. In this work the pigment photobleaching has been studied on PSII core complexes, by observing the effects of different factors such as the aggregation state (PSII monomers and dimers were used), temperature (20 °C and 10 °C temperatures were tested) and the presence of the exogenous phospholipids (cardiolipin and phosphatidylglycerol). In particular, PSII resistance against white light stress was studied by means of UV/VIS Absorption and Fluorescence Emission measurements. It was found that PSII dimers resulted more resistant against photobleaching and that lower temperature reduces the pigment photodestruction. Moreover, the presence of phosphatidylglycerol or cardiolipin enhanced the PSII resistance to the photobleaching phenomenon, mainly at lower temperatures.

1. Introduction

It is widely proved that prolonged exposure of photosynthetic oxygen evolving membranes leads them to an evident loss in the photosynthetic activity, which is commonly known as photoinhibition [1]. This kind of photodamage, which targets mainly the Photosystem II (PSII) protein, is due to the incapability of photosynthetic proteins to convert all the exceeding energy into electrochemical energy by the electron transfer: therefore, under strong illumination and aerobic conditions, the excess in energy brings about the formation of singlet oxygen molecules, which in PSII is responsible for the damage of the D1 polypeptide, as well as of the pigments present as cofactors in the protein scaffolds [2,3]. The primary target for the singlet oxygen is not known for sure, but some experimental evidences showed that this species preferentially destroys the P680 chlorophyll [4] by oxidative disruption of the π-electron system [5]. Then, the singlet oxygen production does not stop after P680 is oxidized, since triplet states are generated in the disconnected antenna systems by intersystem crossing [6]. The photodisruption of photosynthetic pigments (commonly chlorophylls and carotenoids) under strong illumination is an important phenomenon which is called photobleaching. Different hypotheses have been proposed about what the main step in photoinhibition consists in [1,5,6], and recent studies assert that the main step of the photoinibition could be attributed to inhibition at the PSII acceptor side, and in particular at Q_A, whose function was found to be impaired because of its double reduction during the early phase of photoinhibition [1,7,8]. The doubly reduced Q_A is an unstable state and it can react with P680 bringing about the formation of P680 triplet, responsible in turn of the production of singlet oxygen [9]. The singlet oxygen formation could occur also by participation of Fe–S centers in thylakoid membranes [10]. It has been proposed that also an additional photoinhibition at the PSII donor side can occur: in this case, the inactivation has been shown to result from impairment of electron transport between the manganese cluster and P680 [1]. Anyway, for both the mechanisms proposed for photoinactivation, although they originate from the acceptor or donor side, the final damage to the reaction center is most probably around the primary electron donor P680 [1].

Recently, it has been demonstrated that also the Photosystem I (PSI) can be photoinhibited, particularly at low temperature in chilling sensitive plants [11]; this inhibition process was found to be more important for the Light Harvesting Complex (LHCI) of PSI than for the PSI core complex [12] and this suggests that the photobleaching could have a key role in the photoinhibition process, as well as temperature.

In general, during the photobleaching process, a loss of bulk photosynthetic pigments occurs and a greater amount of the excess energy reaches the holochromes, which are the pigment cofactors of the proteins, located at the end of the energy migration pathways; therefore, the holochromes undergo faster photobleaching. As reported, the chlorophyll aggregates which absorbs light at longer wavelengths, bleach

* Corresponding author. Dipartimento di Chimica, Università degli Studi di Bari, Via Orabona 4, 70126 Bari, Italy. Tel.: +39 080 5443443; fax: +39 080 5442128.
E-mail address: catucci@chimica.uniba.it (L. Catucci).

1567-5394/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2008.04.007

before the holochromes absorbing at shorter wavelengths [13,14], and the pigments of PSI submembrane fractions bleach slower than those of PSII complex, the latter being more sensitive towards photoinhibition, as reported by Miller and Carpentier [15].

The photobleaching process can be enhanced in the presence of compounds which inhibit the photosynthesis, such as herbicides interacting at the PSII Q_B side [16]; however, the photobleaching can also be avoided by adding compounds which act as singlet oxygen scavengers, such as histidine and rutine [6], or anti-radical species like α-tocopherol, superoxide dismutase, ascorbate, flavonols and carotenoids [15], thus confirming the key role of oxygen in the photoinhibition and photobleaching phenomena. In general, different factors can be involved in the pigment photobleaching.

In this work, functionally active PSII core monomers and dimers were previously isolated, and then the effects of their aggregation state, of temperature and of phospholipids (in particular phosphatidylglycerol and cardiolipin) on PSII pigment photobleaching were studied. It is interesting to carry out investigations on the role of phospholipids in the PSII stabilization against light excess, since data in the literature show that lipid environment is very critical in fixing the biophysico-chemical properties of many intrinsic proteins [17]. In particular, it has been documented that phosphatidylglycerol (PG) plays specific roles in the electron transport at the Q_B-binding site in PSII complexes and in their dimerization processes [18,19]; moreover, it has been recently reported that cardiolipin (CL) content is enriched in the purified PSII complexes and CL content per reaction center was found to be double in PSII dimers than in monomers [20]. Furthermore, studying the effect of the PSII aggregation state is attractive because data report that the dimer state is more functional and stable than its monomeric counterpart [21]: these observations could give rise to the question if PSII dimer pigments are more resistant against photobleaching than monomer ones. Also the temperature is an important factor to consider, since for thylakoid membranes lowering the temperature can avoid almost completely the pigment photobleaching [16], and since it was found that PSI can be photoinhibited especially at low temperature in some chilling sensitive plants [22].

2. Materials and methods

2.1. Materials

Triton X-100, *n*-dodecyl-β-D-maltoside (DM), *n*-octyl-β-D-glucopyranoside (OG), [2-*N*-morpholine]ethane-sulphonic acid, NaCl, $CaCl_2$, $NaHCO_3$ were purchased from Sigma; acetone (99.8%) and sulphuric acid (96%) were purchased from C. Erba; 2,5-dichloro-*p*-benzoquinone was purchased from Kodak. Lipid standards, dipalmitoyl phosphatidylcholine (DPPC), PG and CL, were purchased from Sigma. In particular, PG ammonium salt from egg yolk lecithin was used, having 34% C(16:0), 2% C(16:1), 11% C(18:0), 32% C(18:1), 18% C (18:2), 3% C(20:3) as acyl chain composition; CL sodium salt from bovine heart was purchased, having 1% C(16:0), 2% C(16:1), 8% C(18:1), 87% C(18:2), 1% C(18:3) as acyl chain composition.

2.2. Isolation of PSII core monomers and dimers

Chloroplasts, thylakoids and membrane fractions enriched in PSII were isolated from market spinach leaves according to Hankamer's procedure [23–26]. Chloroplasts were obtained from spinach leaves using differential centrifugation and thylakoids from chloroplasts by their rupture in a hypotonic solution. Thylakoids were solubilized with Triton X-100 in order to extract PSII-enriched membranes (BBYs). These last membranes were solubilized with OG to detach the LHCII proteins from the PSII core (OG-core complexes). OG-core complexes were then diluted with an aqueous buffer (MNCB) containing [2-*N*-morpholine]ethane-sulphonic acid (25 mM), NaCl (10 mM), $CaCl_2$ (5 mM) and $NaHCO_3$ (10 mM), and DM was added to the solution, in order to obtain a chlorophyll (Chl) concentration of 0.5 mg/ml and a 25 mM DM concentration. Sucrose gradients, supplemented with DM (0.03%, w/v), were employed to obtain oxygen evolving PSII core monomers and dimers which lack the 23 and 17 kDa extrinsic proteins and the CP29, CP26, CP24 chorophyll binding proteins. The concentration of PSII complex samples was estimated as Chl mg/ml [27] and adjusted to 0.015 mg/ml for all the measurements in this work. Oxygen evolution rates were obtained by means of a composite oxygraphic device by Rank Brothers. Values of about 400 μmol O_2/h mg chl were obtained for freshly prepared PSII complexes.

2.3. High light treatment

The illumination of isolated PSII core monomers and dimers was conducted in temperature-controlled vessel under continuous stirring, at 20 °C or 10 °C. Fluorescence emission and absorption measurements were carried out during illumination at different sampling times.

The PSII suspensions were exposed to heat filtered white light by means of an optical fibre lamp at 150 W (lamp temperature 3300 K), under aerobic conditions, for 20 min. In the experiments for investigations on the effects of phospholipids, PG and CL were added to PSII solutions before illumination: the obtained lipid/PSII ratio (mol/mol) was about 100, in accordance with the experimental observations by Kruse et al. [28].

2.4. Steady-state fluorescence and absorption measurements

Fluorescence measurements were carried out using a Varian Cary Eclipse spectrofluorimeter. Visible absorption spectra were recorded using a Varian CARY/3 spectrophotometer using 1 cm path length quartz cells.

2.5. Statistical analysis

The GraphPad InStat software (Sigma, St. Louis, MO) was used to process the data by analysis of variance (ANOVA) to indicate statistically significant differences between means (one-way ANOVA with post-hoc Tukey test, $p<0.05$).

All reported data represent mean values (standard deviation obtained from three replicates).

3. Results and discussion

3.1. Photobleaching of PSII monomer core complex

In Fig. 1, the visible absorption spectra of untreated PSII monomer and of PSII monomer after 20 min of constant white light illumination at 20 °C are reported. The spectra show typical absorption peaks at 675 and 436 nm due to chlorophyll *a* and shoulders in the range 450–550 nm attributable to carotenoids [29–33]. It can be confirmed that the absorption intensities at 436 and 675 nm decrease with time of irradiation, as it was expected.

The rate of intensity decrement for the 675 nm was found to be similar to the one for the 436 nm peak; actually, the A_{675}/A_{436} ratio was found to be equal to 0.73 at zero time and to 0.70 after 20 min of constant illumination. As it can be noticed, the shoulder at about 550 nm, typical for carotenoids, almost completely disappears after 20 min of light stress. These information agree with data in the literature, which in particular assert that long wavelength absorbing chlorophylls and carotenoids are the first species to be influenced by strong light irradiation under aerobic condition [6]. In addition, the position of the peak in the red region changes from 675 nm at zero time, to 673 nm after 20 min of illumination; a blue shift can also be pointed out for the blue region peak, which is at 436 nm at zero time, and at 435 nm after 20 min of light stress. These blue shifts are in

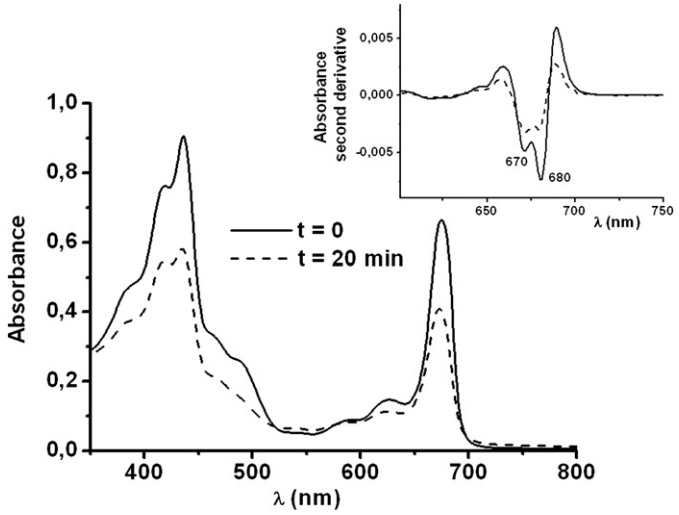

Fig. 1. Effect of illumination on PSII monomer absorption spectra at 20 °C: PSII monomer absorption spectra at zero time (continuous line) and after 20 min of constant white light illumination (dashed line). The inset shows the second derivatives absorption spectra in the red region.

accordance with data previously reported in the literature and could be due in part to the loss of coupling between chlorophylls, and in part to the fact that chlorophylls absorbing at longer wavelengths are bleached first than others [15,34]. This is confirmed by analysis of the second derivative absorption spectra in the red region reported in the inset of Fig. 1. As it can be observed two peaks contribute to the main absorption band at 675 nm, the peak at 680 nm, considered mostly due to P680 and the peak at 670 attributed to peripheral chlorophyll [35,36]. It is evident that the decrease at 680 nm was more pronounced than that at 670 nm after light treatment.

In Fig. 2 it is possible to observe the time evolution of steady-state emission spectra of PSII monomer complexes for a period of time of 20 min under constant illumination at 20 °C: emission spectra show a typical band at 683 nm and a shoulder at 740 nm, which probably originated from a vibrational sublevel as indicated in literature [37–39]. A decrease in the fluorescence emission intensity is evident under light irradiation. As reported in a previous paper [40], PSII complexes in aqueous buffer and in the absence of sucrose can be involved in an aggregation process, which causes a decrease in their chlorophyll fluorescence emission intensity (fluorescence emission quenching) which is not correlated to the photobleaching process. In order to exclude that the emission intensity reduction was due to PSII aggregation processes, also PSII monomers emission in dark conditions was checked. However, it has to be considered that PSII samples had been treated with sucrose which is known to preserve photosynthetic proteins, also from aggregation processes [21]. In the inset of Fig. 2, the PSII emission intensity decrement percentages (at peak value) are reported as function of time in dark or light conditions at 20 °C; the decrements are all normalized with respect to the zero-time intensity value, as for all the measurements reported afterwards in this paper. Although a small decrement in the emission intensity was recorded for untreated samples, the decrease under light conditions was much faster than in dark conditions; it can be concluded that the emission intensity decrement showed in Fig. 2 can be mostly attributed to the light stress.

All these observations confirm that the strong illumination brings about a time dependent-destruction of the pigments present in the PSII monomers; this photobleaching is a photooxidative process caused by the protein incapability to convert the excessive energy by the electron transport, and the relevant photodamage is reported to be irreversible [1].

3.2. Effect of PSII aggregation state

It is widely demonstrated that PSII can exist *in vivo* as both a monomeric and a dimeric protein complex; evidences suggest that the dimeric form is more functional and stable than the monomeric one [21]. Here the photobleaching process has been observed both for PSII monomers and dimers, in order to check any differences due to the protein aggregation state.

In Fig. 3 the emission decrements at peak value (683 nm) are reported for PSII monomers and dimers as a function of the illumination time (20 min) at 20 °C; the intensity values are percentages normalized with respect to the value at zero time for each sample. The same experiment was monitored by visible absorption giving rise to values of percentage absorbance (at 675 nm) equal to 61.6% for monomers and to 79.5% for dimers after 20 min of illumination. A decrement in both fluorescence and absorption intensity can be observed for PSII monomers and dimers, but dimer pigments appear to be more resistant and stable than monomer ones against the strong illumination. In particular the P680 seems to be better preserved in dimer complexes as suggested by the second derivative absorption spectra reported in the inset of Fig. 3 in which a lower decrease of the band at 680 nm for the dimer with respect to that of monomer is observed after light treatment. The reduced photobleaching of the long wavelength absorbing

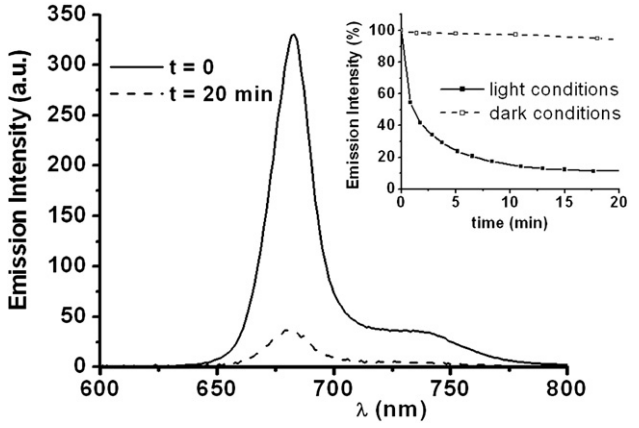

Fig. 2. Effect of illumination on PSII monomer fluorescence emission spectra at 20 °C: PSII monomer emission spectra at zero time (continuous line) and after 20 min of constant white light illumination (dashed line). The inset shows the emission intensity % at 683 nm as a function of time under light (continuous line, full squares) and dark conditions (dashed line, empty squares) for PSII monomers (lines are guides to read the graph). The excitation wavelength is 436 nm.

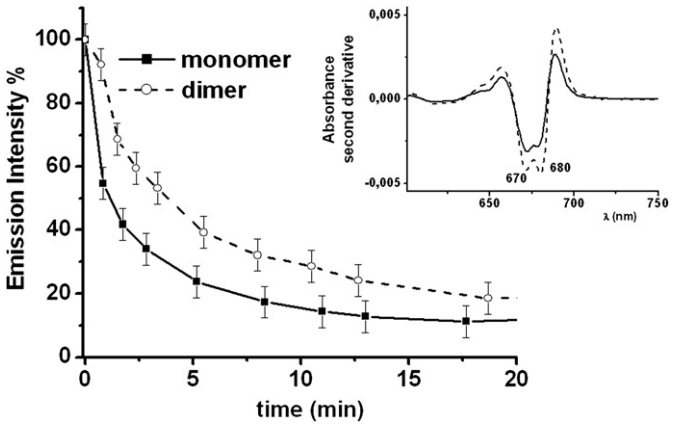

Fig. 3. Emission intensity % at 683 nm as a function of time for PSII monomers (continuous line, full squares) and PSII dimers (dashed line, empty circles) under constant white light illumination at 20 °C. Lines are guides to read the graph. The inset shows the second derivatives of the PSII monomer and dimer absorption spectra, recorded after 20 min of constant white light illumination. The excitation wavelength is 436 nm.

Table 1
Ratios of absorbance second derivatives at 680 and 670 nm for PSII monomers and dimers at zero time and after 20 min of strong white light illumination

PSII samples		d'' 680 d'' 670	
		20°C	10°C
Monomer	$t=0$	1.57	1.74
	$t=20'$	0.95	1.11
Dimer	$t=0$	1.67	1.72

chlorophylls accounts also for the observed reduced blue shift (1 nm) of the absorption band at 675 nm observed for treated dimer compared to monomer which presents a shift of 2 nm of this red absorption band when illuminated with strong light for 20 min. Since it has been reported [41] that inhibition of electron transport does accelerate the pigment bleaching process in photosynthetically active material, the higher integrity of both donor and acceptor side, as well as, the higher P680 stability recorded for PSII dimer complexes could account for the slower photobleaching observed for these complexes.

3.3. Effect of temperature

The temperature dependency of pigment photobleaching was also studied. In particular lowering the temperature from 20 °C to 10 °C reduced absorption and emission spectra changes, recorded after illumination of samples. The temperature effect was more pronounced for PSII monomer than dimer. The absorption decrease, in fact, equal in the blue and in the red region for both PSII complexes, passed from 38.4% to 21.8% and from 20.5% to 13.4% when monomer and dimer respectively were illuminated at 10 °C. At this lower temperature P680 seems to be stabilized both in monomer and dimer complexes, as suggested by the higher second derivative absorption ratio values (d_{680}''/d_{670}'') reported in Table 1. According to the absorption data reduced emission decrement were mainly observed for monomer samples illuminated at 10 °C (data not shown).

3.4. Effect of lipids

Subsequently, the effect of the phospholipids CL or PG on PSII pigment photobleaching phenomenon was investigated. As reported in the material section, PG ammonium salt from egg yolk lecithin was used, having 34% C(16:0), 2% C(16:1), 11% C(18:0), 32% C(18:1), 18% C (18:2), 3% C(20:3) as acyl chain composition; CL sodium salt from bovine heart was also tested, having 1% C(16:0), 2% C(16:1), 8% C(18:1), 87% C(18:2), 1% C(18:3) as acyl chain composition.

It should be noted that adding phospholipids could enhance PSII aggregation processes, as recently reported by our group [42]: this phenomenon could produce a progressive Resonance Light Scattering increment and an emission decrement due to chlorophyll quenching [43], that could slightly interfere with the emission changes due to photobleaching. Even if this interfering effect can be considered as negligible, in order to slow down these protein aggregative processes, thus reducing the consequential emission quenching and minimizing interferences for the photobleaching experiments, PSII samples were used with sucrose as osmo-protectant.

In the photobleaching experiments here reported, the intensitiy and the absorbance percentages were calculated with respect to initial values: in Fig. 4a, the emission percentages (at 683 nm) after 20 min with respect to the zero-time values under strong illumination are reported for PSII monomers or dimers in the absence of exogenous lipids and in the presence of PG or CL at 20 °C; the percentages are averages of three replicates. No particular effect on monomer photobleaching rates can be noticed by these experiments. This effect on PSII monomers seems to be more evident in the presence of PG. For dimers no effect was observed in presence of phospholipids CL or PG.

3.5. Effect of lipids and temperature

In order to investigate the concomitant effects of phospholipids and temperature, the measurements were repeated at 10 °C both for monomers and dimers.

From Fig. 4b it is clear that lowering the temperature enhances the stabilizing effect of PG with respect to PSII monomer photobleaching, since the emission intensity decrement under strong illumination appears to be slowed down in the presence of this particular lipid. These observations are confirmed by the absorption measurements (data not shown). No evident effect was pointed out in the presence of CL, even at 10 °C.

For PSII dimers, stabilizing effects were noticed in the presence of both CL or PG, but the latter resulted to stabilize more evidently the PSII complexes against the photobleaching.

It can be hypothesized that PG and CL could act as oxygen scavengers, thanks to their double bonds, this explaining their stabilizing effects with respect to PSII complexes at low temperatures. In order to make this hypothesis stronger, the emission experiments were repeated by adding a fully saturated lipid (DPPC) to PSII complexes (monomers or dimers) at 20 or 10 °C (data not shown).

In every tested case it was found that the residue PSII complex emission percentage in the presence of DPPC was less than in the absence of exogenous lipids: for instance, for dimers at 10 °C, i.e. in the conditions in which the lipids effects were found to be the most evident, the residue PSII emission percentage was 15.41%, that is a value smaller than for PSII in the presence of the other tested lipids. Therefore, since in the presence of unsaturated phospholipids the emission of PSII complexes was found to be better preserved, it can be hypothesized that PG and CL double bonds can be involved in the oxygen scavenging.

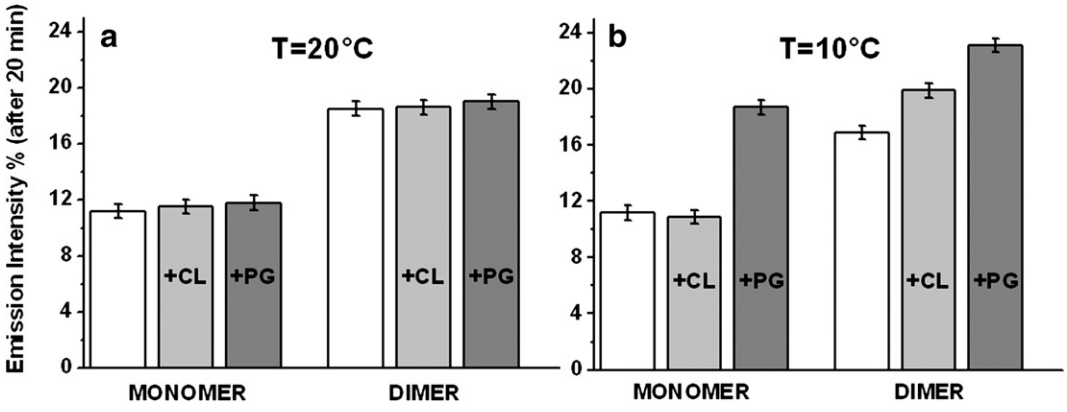

Fig. 4. PSII monomer and dimer emission intensity percentages after 20 min of constant white light illumination at 20 °C (panel "a") or at 10 °C (panel "b"), in the absence or in the presence of PG or CL. The excitation wavelength is 436 nm.

4. Conclusions

The results obtained in this work showed that the PSII pigment photobleaching phenomenon, caused by the exposition of the complexes to strong constant illumination, could depend on the aggregation state of the protein themselves. Actually, it was found by fluorescence emission and absorption measurements that the PSII dimer pigments were bleached more slowly than the monomer ones, and also the residue fluorescence emission intensity and absorption after 20 min of white light exposition were higher for PSII dimer pigments at 20 °C. These data are in accordance with results previously reported in the literature, according to which PSII dimeric state is a more functional and active form than the monomeric one [21], and it is possible that this affect also the protein pigment photobleaching. The effect of adding phospholipids PG and CL was also checked, and as a result it was found that the PSII emission intensity was only slightly better preserved in the presence of one of these two lipids at 20 °C, the effect being more evident in the presence of PG, for both PSII monomer and dimer pigments. Moreover, the consequence of lowering the temperature was investigated, by repeating all the experiments at 10 °C: the residue emission intensity after 20 min of high-light stress was found to be interestingly higher in the presence of the phospholipids, in particular of PG, for both PSII monomer and dimer pigments at 10 °C. The fact that the presence of PG or CL may improve the PSII resistance against strong illumination could be due to the mechanism of PSII photoinhibition and in particular to the important role played by singlet oxygen molecules: it is possible that PG and CL can act like oxygen scavengers by being easily oxidized by the singlet oxygen molecules generated under high-light conditions, as reported for different lipids and fatty acids in the literature [34,43,44]. In fact, the lipids are propense to be oxidized by oxygen, and the reaction is known to be favoured in the presence of singlet oxygen molecules. In particular, singlet oxygen can add to the double bonds of polyunsaturated fatty acid chain producing lipid peroxides [44,45]. This hypothesis was also enforced by further tests in which a fully saturated lipid (DPPC) was added to PSII complexes, bringing about no significant effect.

The synergic effect of lowering the temperature and adding PG or CL could cause the oxygen species to move slower than at 20 °C, and this effect should be more evident if the PSII complexes were embedded in the thylakoid membranes, since the membrane fluidity is strongly dependant on the temperature. In fact, it has been reported [16] that lowering the temperature to 4 °C can almost completely protect the protein pigments from photobleaching in spinach thylakoid membranes: several hypotheses were done to explain this effect, and the most plausible of them consisted in the fact that at 4 °C the mobility of the oxygen molecules is drastically reduced, especially across the membrane system and therefore, for the oxygen it is more difficult to reach the electron transport chain and to produce singlet oxygen molecules able to destroy the pigments and to damage the D1 subunit [16]. Our results seem to confirm this hypotheses, since although the isolated PSII core complex photobloeaching was not found to be strongly dependant on the temperature, after the addition of phospholipids PG or CL, the temperature appeared to influence the photosynthetic pigment resistance against the high-light stress.

References

[1] E.-M. Aro, I. Virgin, B. Anderson, Photoinibition of Photosystem II. Inactivation, protein damage and turnover, Biochim. Biophys. Acta 1143 (1993) 113–134.
[2] D.J. Kyle, I. Ohad, C.J. Arntzen, Membrane protein damage and repair: selective loss of a quinone-protein function in chloroplast membranes, Proc. Natl. Acad. Sci USA 81 (1984) 4070–4074.
[3] I. Ohad, D.J. Kyle, C.J. Arntzen, Membrane protein damage and repair: removal and replacement of inactivated 32-kilodalton polypeptides in chloroplast membranes, J. Cell. Biology 99 (1984) 481–485.
[4] A. Telfer, W.-Z. He, J. Barber, Spectral resolution of more than one chlorophyll electron-donor in the isolated photosystem-II reaction center complex, Biochim. Biophys. Acta 1017 (1990) 143–151.
[5] A.A. Krasnovskii, Photoluminescence of singlet oxygen in pigment solutions, Photochem. Photobiol. 29 (1979) 29–36.
[6] N.P. Mishra, C. Francke, H.J. van Gorkom, D.F. Ghanotakis, Destructive role of singlet oxygen during aerobic illumination of the photosystem II core complex, Biochim. Biophys. Acta 1186 (1994) 81–90.
[7] I. Setlik, S.I. Allskhverdiev, L. Nedbal, E. Setlikova, V. Klimov, Three types of photosystem II pholoinactivation. I Damaging processes on the acceptor side, Photosynth. Res. 23 (1990) 39–48.
[8] I. Vass, S. Syring, T. Hundal, A. Koivuniemi, E.-M. Aro, B. Andersson, Reversible and irreversible intermediates during photoinhibition of photosystem II: stable reduced Q_A species promote chlorophyll triplet formation, Proc. Natl. Acad. Sci., USA 89 (1992) 1408–1412.
[9] C.H. Foyer, M. Lelandaism, K.J. Kunert, Photooxidative stress in plants, Physiol. Plant. 92 (1994) 696–717.
[10] J. Jung, H.-S. Kim, The chromophores as endogenous sensitizers involved in the photogeneration of singlet oxygen in spinach thylakoids, Photochem.Photobiol. 52 (1990) 1003–1009.
[11] K. Sonoike, Selective photoinhibition of photosystem I in isolated thylakoid membranes from cucumber and spinach, Plant Cell. Physiol. 36 (1995) 825–830.
[12] Y. Hui, J. Wei, R. Carpentier, Degradation of the photosystem I complex during photoinhibition, Photochem. Photobiol. 72 (2001) 508–512.
[13] J.B. Thomas, M.H.M. Bollen, W.J. Klijn, Photobleaching and dark bleaching of *Euglena gracilis* chloroplast fragments, Acta Bot.Neerl. 25 (1976) 361–369.
[14] G. Zucchelli, F.M. Garlaschi, R.C. Jennings, Spectroscopic analysis of chlorophyll photobleaching in spinach thylakoids, grana and light harvesting chlorophyll *a*/*b* protein complex, J. Photochem. Photobiol.Biol. 2 (1988) 483–490.
[15] N. Miller, R. Carpentier, Energy dissipation and photoprotection mechanisms during chlorophyll photobleaching in thylakoid membranes, Photochem.Photobiol. 54 (1991) 465–472.
[16] M.Y. Velitchkova, R. Picorel, Photobleaching of photosynthetic pigments in spinach thylakoid membranes. Effect of temperature, oxygen and DCMU, Biophys. Chem. 107 (2004) 25–32.
[17] L. Catucci, N. Depalo, V.M.T. Lattanzio, A. Agostiano, A. Corcelli, Neosynthesis of cardiolipin in *Rhodobacter sphaeroides* under osmotic stress, Biochemistry 43 (2004) 15066–15072.
[18] O. Kruse, B. Hankamer, C. Konczak, C. Gerle, E. Morris, A. Radunz, G.H. Schmid, J. Barber, Phosphatidylglycerol is involved in the dimerization of Photosystem II, J. Biol. Chem. 275 (2000) 6509–6514.
[19] I. Sakurai, J.-R. Shen, J. Leng, S. Ohashi, M. Kobayashi, H. Wada, Lipids in oxygen-evolving photosystem II complexes of cyanobacteria and higher plants, J. Biochem. 140 (2006) 201–209.
[20] N. Depalo, L. Catucci, A. Mallardi, A. Corcelli, A. Agostiano, Enrichment of cardiolipin content throughout the purification procedure of photosystem II, Bioelectrochemistry 63 (2004) 103–106.
[21] L. Catucci, W. Dörner, J. Nield, B. Hankamer, I. Vass, J. Barber, Isolation and characterisation of oxygen evolving Photosystem II core complexes from spinach in the presence of glycine betaine, in: G. Garab (Ed.), Photosynthesis: Mechanisms and Effects, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1998, pp. 973–976.
[22] K. Sonoike, Selective photoinhibition of Photosystem I in isolated thylakoid membranes from cucumber and spinach, Plant Cell.Physiol. 36 (1995) 825–830.
[23] E.J. Boekema, B. Hankamer, D. Bald, J. Kruip, J. Nield, A.F. Boonstra, J. Barber, M. Rogner, Supramolecular structure of the photosystem II complex from green plants and cyanobacteria, Proc. Natl. Acad. Sci. USA 92 (1995) 175–179.
[24] D.A. Berthold, G.T. Babcock, C.F. Yocum, A highly resolved oxygen-evolving photosystem II preparation from spinach thylakoid membranes: EPR and electron transport properties, FEBS Lett. 134 (1981) 231–234.
[25] M. Miyao, N. Murata, Calcium ions can be substituted for the 24-kDa polypeptide in photosynthetic oxygen evolution, FEBS Lett. 168 (1984) 118–120.
[26] N. Murata, M. Miyao, T. Omata, H. Matsunami, T. Kuwabara, Stoichiometry of components in the photosynthetic oxygen evolution system of photosystem II particles prepared with Triton X-100 from spinach chloroplasts, Biochim. Biophys. Acta 765 (1984) 363–369.
[27] D.I. Arnon, Copper enzymes in isolated chloroplasts. Polyphenol-oxidase in *Beta vulgaris*, Plant Physiol. 24 (1949) 1–15.
[28] O. Kruse, B. Hankamer, C. Konczak, C. Gerle, E. Morris, A. Radunz, G.H. Schmid, J. Barber, Phosphatidylglycerol is involved in the dimerization of Photosystem II, J. Biol. Chem. 275 (2000) 6509–6514.
[29] J. Wang, J. Shan, Q. Xu, X. Ruan, Y. Gong, T. Kuang, N. Zhao, Spectroscopic study of trypsin, heat and Triton X-100-induced denaturation of the chlorophyll-binding protein CP43, J. Photochem.Photobiol., B: Biol. 58 (2000) 136–142.
[30] I. Enami, K. Kamino, J. Shen, K. Satoh, S. Katoh, Isolation and characterization of Photosystem II complexes which lack light-harvesting chlorophyll a/b proteins but retain three extrinsic proteins related to oxygen evolution from spinach, Biochim. Biophys.Acta 977 (1989) 33–39.
[31] M. Germano, A.Y. Shkuropatov, H. Permentier, R. de Wijn, A.J. Hoff, V.A. Shuvalov, H.J. van Gorkom, Pigment organization and their interactions in reaction centers of photosystem II: optical spectroscopy at 6 K of reaction centers with modified pheophytin composition, Biochem. 40 (2001) 11472–11482.
[32] X. Ruan, D. Li, Q. Xu, H. Mao, G. Li, Y. Gong, T. Kuang, N. Zhao, Phosphatiylcholine-induced reactivation of photosystem II membranes pretreated with Triton X-100, J. Photochem.Photobiol., B: Biol. 67 (2002) 109–115.
[33] V. Masters, P. Smith, E. Krausz, R. Pace, Stark shifts and exciton coupling in PSII 'Supercores', J. Lumines 94–95 (2001) 267–270.
[34] S. Rajagopal, R. Carpentier, Retardation of photo-induced changes in Photosystem I submembrane particles by glycinebetaine and sucrose, Photosynth. Res. 78 (2003) 77–85.
[35] J.P. Dekker, R.V. Grondelle, Primary charge separation in photosystem II, Phosynth. Res. 63 (2000) 195–208.

[36] S. Liu, F.Q. Dong, C.Q. Tang, T.Y. Kuang, L.B. Li, Y.L. Liu, Photodemage to pigment in the photosystem II reaction center D1/D2/cytochrome *b*559 complex, J. Integrat. Plant Biology, 48 (2006) 800–806.
[37] F. Franck, P. Juneau, R. Popovic, Resolution of the Photosystem I and Photosystem II contributions to chlorophyll fluorescence of intact leaves at room temperature, Biochim.Biophys.Acta 1556 (2002) 239–246.
[38] O. Björkman, B. Demming, Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins, Planta 170 (1987) 489–504.
[39] B.D. Hsu, J.Y. Lee, Fluorescence quenching by plastoquinone in an oxygen-evolving photosystem-II-enriched preparation, J.Photochem.Photobiol.,B: Biol. 30 (1995) 57–61.
[40] A. Ventrella, L. Catucci, V. Villari, L. Monsù Scolaro, A. Agostiano, Focus on the aggregation processes of Photosystem II complexes, Bioelectrochem. 70 (2007) 33–38.
[41] R. Carpentier, Influence of high light intensity on photosynthesis: photoinhibition and energy dissipation, in: M. Pessarakli (Ed.), Handbook of Photosynthesis, Dekker, New York, 1997, pp. 443–450.
[42] A. Ventrella, L. Catucci, G. Mascolo, A. Corcelli, A. Agostiano, Isolation and characterization of lipids strictly associated to PSII complexes: focus on cardiolipin structural and functional role, Biochim. Biophys. Acta 1768 (2007) 1620–1627.
[43] A. Ventrella, L. Catucci, V. Villari, L. Monsù Scolaro, A. Agostano, Focus on the aggregation processes of Photosystem II complexes, Bioelectrochem. 70 (2007) 33–38.
[44] H.K. Ledford, K.K. Niyogi, Singlet oxygen and photo-oxidative stress management in plants and algae, Plant, Cell and Environment 28 (2005) 1037–1045.
[45] A.W. Girotti, Lipid hydroperoxide generation, turnover, and effector action in biological systems, J. Lip. Res. 39 (1998) 1529–1542.