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Photoinhibitory light-induced changes in the structure and function of isolated Photosystem I submembrane particles

S Rajagopal[1], NG Bukhov[1, 2], R Carpentier[1*]

[1]*Groupe de Recherche en Énergie et Information Biomoléculaires, Université du Québec à Trois-Rivières, C.P. 500 Trois-Rivières, Québec, Canada, G9A 5H7.*

Fax:1-819-376-5057. E-mail: robert_carpentier@uqtr.uquebec.ca

[2]*K.A. Timiriazev Institute of Plant Physiology, Russian Academy of Science, Botanicheskaya 35, 127276 Moscow, Russia.*

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Introduction

In eukaryotic photosynthetic organisms (algae and plants), the photosystem (PS) I complex is composed of a light harvesting complex I (LHCI) and a core component (CCI). LHCI serves as an accessory antenna to harvest light and funnel its energy to the primary PSI donor, P700, located in CCI. In addition to P700, the CCI complex contains photosynthetic pigments and five different electron carriers, which are needed to carry out the electron transfer occuring in PSI. In general, light constitutes the driving force required for photosynthesis. However, excessive light causes over-saturation of the photosynthetic reaction centers, which eventually results in photoinactivation of the photosynthetic apparatus (Aro *et al*, 1993). Of the two photosystems of oxygenic photosynthesis, PSII is known to be more sensitive than PSI (Aro *et al*, 1993). Hence, photoinhibition can be induced by two mechanisms developing either on the acceptor or donor side of PSII (Aro *et al* 1993).

However, several *in vitro* studies stated that PSI and PSII of isolated chloroplasts are equally susceptible to photoinhibition under strong light. Very recently, the vulnerability of PSI core complexes to strong light at room temperature have been shown to bring about specific damage to LHCI and degradation of reaction center and acceptor side proteins (Hui *et al*, 2000). In *Cucumis sativus* leaves, PSI was photoinactivated by weak light at chilling temperatures, while practically no damage to PSII was observed (Terashima *et al*, 1994). The photoinactivation of PSI was assumed to proceed through three steps: (1) the inactivation of the acceptor side, (2) the destruction of reaction center chlorophylls, and (3) the degradation of the reaction center subunits (Sonoike and Terashima, 1994). To date nothing is known about the photoinactivation of PSI submembrane particles under excessive irradiation *in vitro* at chilling temperatures, which are known to favor PSI damage in intact leaves. Here, we present the profile of changes in PSI submembrane particles illuminated for various periods of time with intense white light (WL) at chilling temperatures (4 ^{o}C).

Materials and Methods

The PSI submembrane particles were isolated from fresh spinach leaves obtained from the local market, according to the procedure of Peters *et al* (1983). In our PSI submembrane preparations the Chl *a*/*b* ratio was found to be higher than 6.0. Isolated preparations (500 μg Chl/mL) were illuminated for various time durations with an intense WL (2000 μE m^{-2} s^{-1}) provided by a 150 W quartz-halogen projector lamp and under continuous stirring at 4 ^{o}C. PSI-mediated electron transfer was measured polarographically. The PSI submembrane proteins were separated by sodium dodecyl sulfate (SDS)-urea polyacrylamide gel electrophoresis (PAGE) after incubating the membranes in the sample buffer containing 6 M urea as described previously in Hui *et al*, (2000). PAGE was carried out in a 13% acrylamide containing 6 M urea. Chorophyll content was determined with 80% acetone.

Results and Discussion

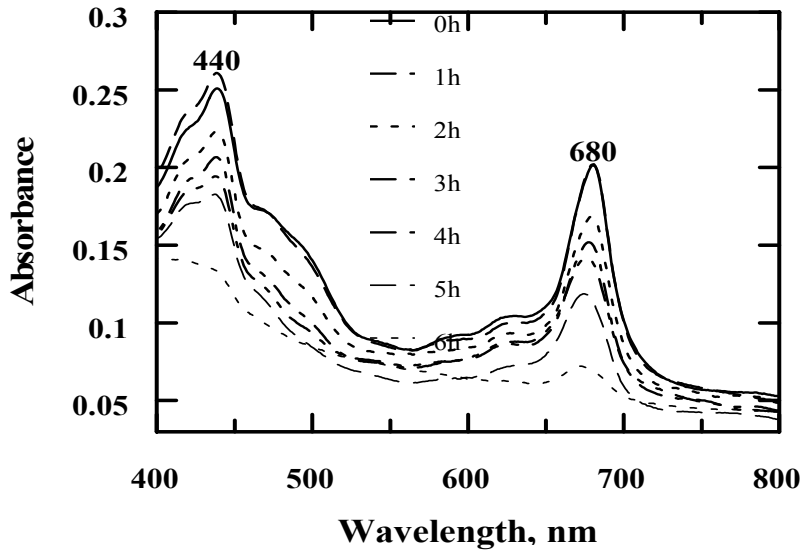

Fig.1. Absorption spectra of the isolated PSI submembrane particles illuminated for various periods of time with intense white light at chilling temperatures (4 ^{o}C).

The earlier reports of Hui *et al*, (2000) has shown that illumination of PSI core complexes with intense WL at room temperatures for short durations altered the photoprotective function of LHCI for the PSI reaction center. Considering the above studies, it was of interest to investigate possible alterations of structure and function of the PSI submembrane particles illuminated at chilling temperatures. Fig.1. shows the absorption spectra of PSI preparations exposed to various periods of illumination in which a 7-nm blue shift of the absorption maximum at 680 nm (Chl *a* absorption) was observed after 5-h illumination. Migration of absorbed energy generally occurs towards holochromes with higher-absorption wavelengths, in which case, Chl forms having absorption maximum in the red at a relatively high wavelength are bleached first. This phonemenon was clearly shown by the blueshift in the absorption maximum at 680 nm in photoinhibited PSI submembrane particles. Further, it was also shown that a shoulder in the absorption spectra at 470 nm due to Chl *b* completely disappeared after 2.5 h of illumination, suggesting that Chl *b* which is bound to LHCI is sensitive to intense WL.

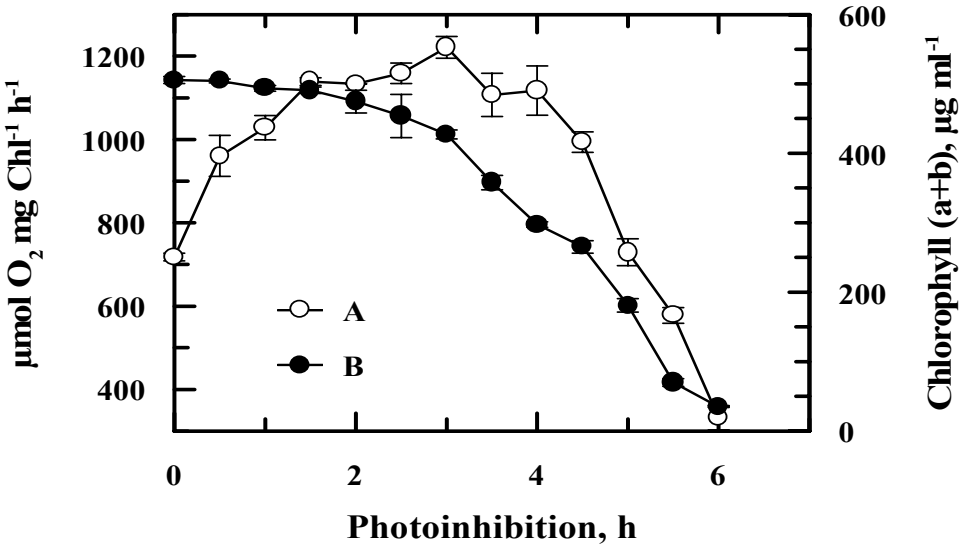

Fig.2. A. Oxygen uptake rates of PSI submembrane particles and B. Total Chl content after various periods of illumination at chilling temperatures.

Fig.2A depicts the O_2 uptake in photoinhibited samples for various illumination periods at chilling temperatures (4 ^oC). The oxygen uptake rates were stimulated up to 4.5 h of illumination and rapidly decreased after 4.5 h. It was suggested that the stimulatory effect on the oxygen uptake rates could be related to the damage on the scavenging system of PSI, such as membrane bound superoxide dismutase or ascorbate peroxidase located near the PS I reaction center. Fig.2B shows the effects of illumination periods on the Chl content in the PSI preparations. The content of total Chl slowly decreased during the first 3 h of illumination but markedly decreased during further illumination. In view of the above absorption spectra and O_2 uptake, the SDS-urea PAGE profile of PSI submembrane particles were anlysed after various illumination periods (Fig.3). The high molecular weight band of the chlorophyll protein complex CP1 gradually disappeared during exposure of isolated particles to intense light. The polypeptides of LHCI with apparent molecular weight of 21-25 kDa are the most sensitive proteins to intense WL at chilling temperatures.

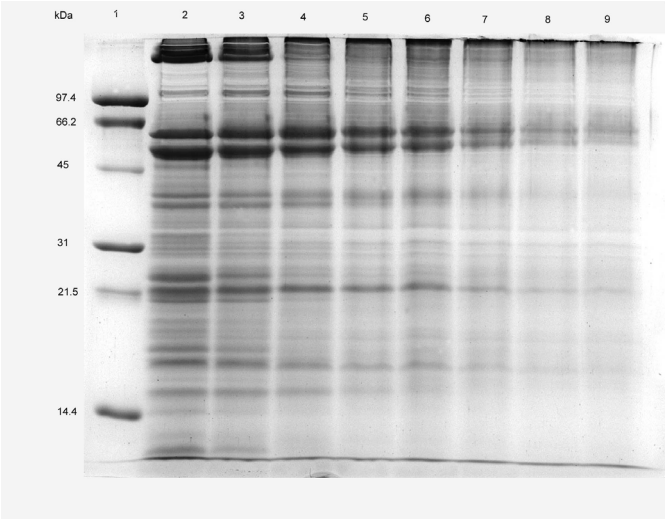

Fig.3. SDS urea PAGE polypeptide profile of PSI submembrane particles after various periods of illumination at chilling temperatures. Lane.1, protein standard; lane 2, 0h; lane 3, 1h; lane 4, 2h; lane 5, 3h; lane 6, 3.5h; lane 7, 4h; lane 8, 4.5h; lane 9, 5h.

LHCI-680 is more sensitive than LHCI-730. However, the PSI-A and PSI-B reaction center proteins are stable during 3.5 h but further photoinhibitory treatment produced a gradual decline of these bands. Several small polypeptides located on the acceptor side of PSI are even more sensitive to intense light at chilling temperatures. Very recently Hui *et al* (2000) reported the degradation of the PSI core complexes during intense WL treatment under room temperature. Thus, our results clearly show that the photoinhibitory treatment of PSI submembranes preparations under chilling temperatures damaged both the reaction center proteins and acceptor-side polypeptides.

Aknowledgements

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