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Heat stress and light stress cooperatively damage the D1 protein in PS II

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Introduction

Plants often suffer from light and heat stresses simultaneously. It was shown previously that heat inactivates PS II at 40 ^{o}C in the dark (Mishra and Singhal, 1992). The heat inactivation was suggested to be due to peroxidation of thylakoid membranes. However, little is known about the relationship between the lipid peroxidation and inhibition of electron transport in PS II. Previously, Nash and co-workers reported that weak red light accelerates heat inactivation of oxygen evolution (Nash et al., 1985). In that study, heat treatment of spinach PS II membranes (47 ^{o}C, 5 min) was shown to induce release of Mn and inactivation of oxygen evolution, which was stimulated by the illumination with weak red light. These results indicate that the inactivation of PS II by the light and heat stresses is due to the donor-side photoinhibition. Here we demonstrated that a mechanism similar to the acceptor-side photoinhibition contributes to the inactivation of PS II under the moderate heat and light stresses.

Materials and Methods

Spinach was purchased at a local market. Leaf-discs of spinach were immersed in 2 mM lincomysin and incubated for 1 hr at PFD of 5 μE m^{-2} s^{-1} at 25 ^{o}C. After dark adaptation for 3 hr , leaf-discs were floated on water in a water-bath (temperature was controlled from 25 to 50 ^{o}C) and illuminated by a tungsten lamp at various PFDs.

PS II activity of the light/heat-treated leaf-discs were measured by a Plant Efficient Analyzer (Hansatech.U.K.). PS II-enriched membranes and thylakoids were prepared from spinach as described previously (Kuwabara and Murata, 1982; Mullet and Chua, 1983). Proteins of the PS II membranes and the thylakoids were analyzed by SDS/urea-PAGE, and the D1 protein and its cross-linked products were detected by immunoblotting with the antibody against the DE loop of the D1 protein.

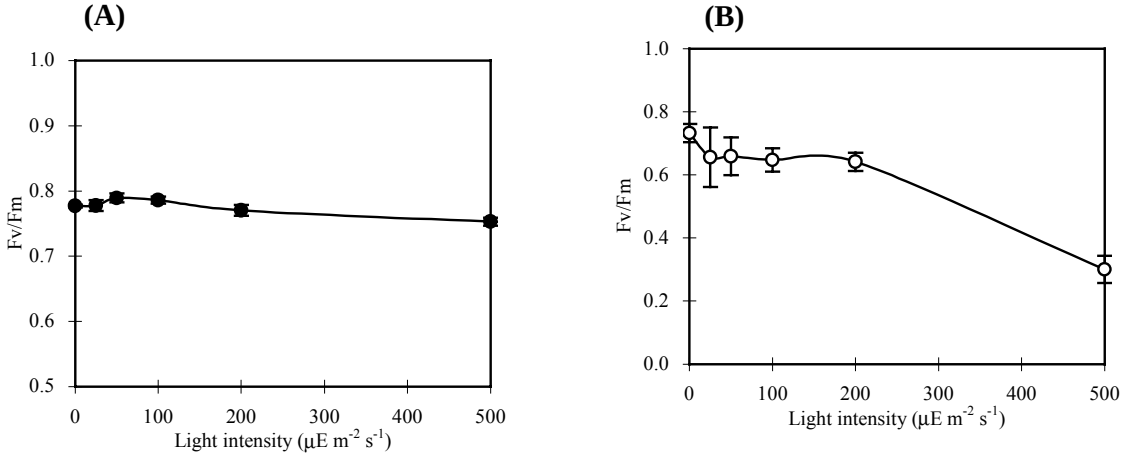

Fig.1. Inactivation of spinach PS II membranes under the illumination at 25^{o}C (A) and 40^{o}C (B). The PSII membranes were illuminated with white light of various light intensities for 10 min. Fv/Fm was measured 3-6 times and the data are means ± S.D.

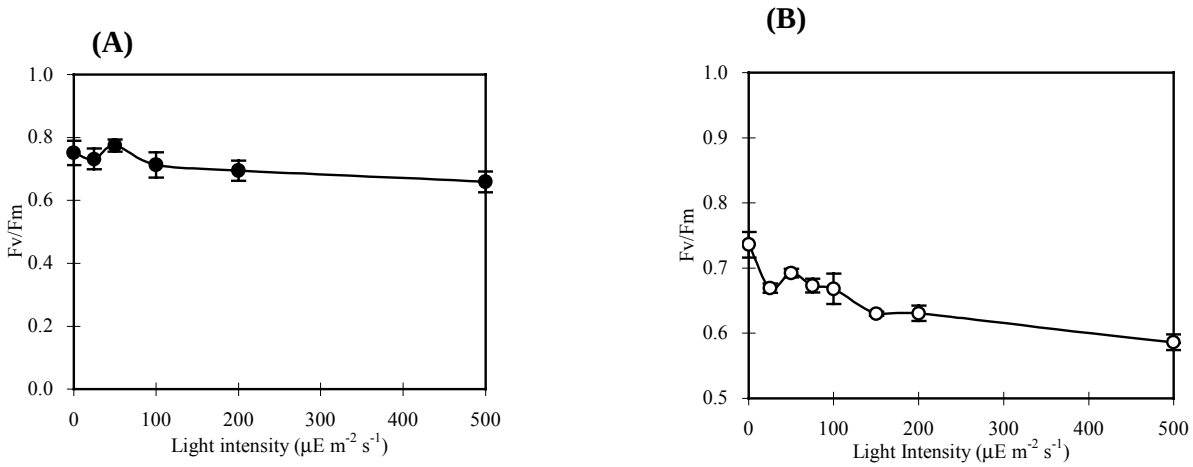

Fig.2. Inactivation of PS II in spinach leaf-disc under the illumination at 25°C (A) and 40°C (B). The leaf-discs were pretreated with lincomysin for 1 hr and dark adapted for 3 hr. Illumination time was 10 min. Fv/Fm was measured 4 times and the data are means ± S.D.

Results

The PS II membranes were incubated at 25 °C or 40 °C at various PFDs for 10min and PS II activity was measured by Fv/Fm of chlorophyll fluorescence (Fig.1A). The PS II activity decreased slightly when light intensity was increased to 500 μE m^{-2} s^{-1} at 25 °C. By contrast, the activity decreased by 8 % with weak illumination of 25 μE m^{-2} s^{-1} at 40 °C (Fig.1B). These results suggest that PS II is easily inactivated by weak light under heat stress conditions. We confirmed these results also in intact thylakoids (data not shown).

To examine whether the cooperative inactivation of PS II by the heat and light stresses takes place in vivo, spinach leaf-discs were incubated at 40 °C under weak light, and PS II activity was measured by Fv/Fm (Fig.2). The leaf-discs were pretreated with lincomysin to inhibit recovery of the PS II activity by newly synthesized D1 proteins. Like in the PS II membranes, the PS II activity in leaf-discs decreased significantly by the illumination with weak light at 40 °C.

The polypeptides of the PS II membranes exposed to weak light at 40°C were separated by SDS/urea-PAGE, and then subjected to Western-blot analysis with the D1-specific antibody (Fig.3). D1/D2 and/or D1/Cytb_{559}-α cross-linked products were detected in all the samples illuminated at 40 °C. These results suggest that the D1 protein is easily damaged by the illumination, and that the damaged D1 protein cross-links with the surrounding proteins under the heat stress conditions. Cross-linked products between the D1 protein and CP43 were not detectable here.

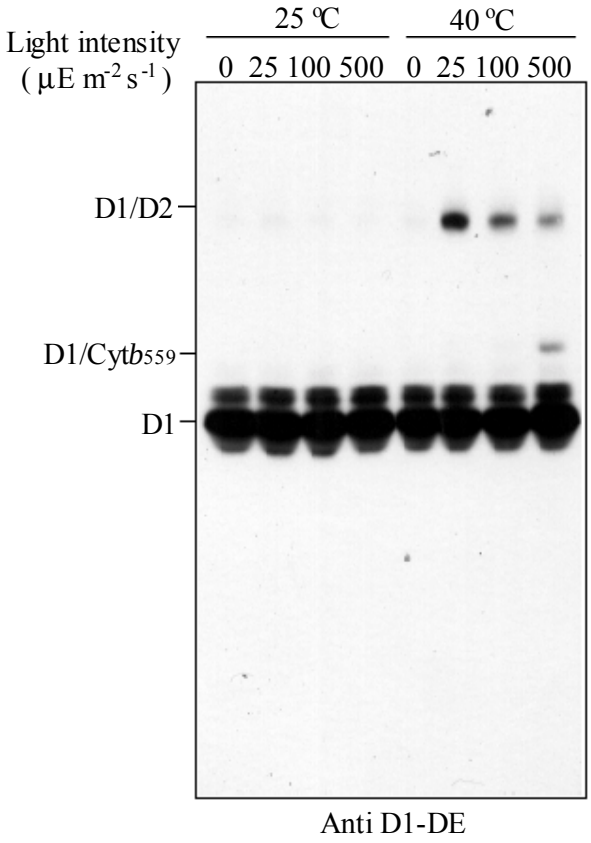

Fig.3. A fluorogram showing the cross-linking of D1 with D2, and D1 with the α-subunit of Cytb_{559}, induced by light and heat treatment of PS II membranes. The PS II membranes were incubated either at 25 or 40°C and illuminated at the light intensity indicated. The proteins were separated by 12.5% SDS-PAGE in the presence of 6M urea, electroblotted onto a PVDF membrane, and immunodetected with the antibody against D1.

Discussion

Our present results demonstrated that PS II is inactivated even by weak light when spinach leaves (or chloroplasts) are treated by moderately high temperature simultaneously. At first, we predicted that heat-stimulated photoinhibition is due to the donor-side photoinhibition, because several events that prevent electron transport at the donor-side of PS II, such as depletion of Cl^-, release of Mn and liberation of OEC subunits, were shown to occur under heat stress conditions (Nash et al., 1985). However, the D1 cross-linked products were observed by the heat/light treatment under the aerobic conditions but not under the anaerobic conditions. It was also observed that the cross-linking of the D1 Protein was suppressed in the presence of the scavengers of oxygen radicals (data not shown). These results suggest that the mechanism of the heat-stimulated photoinhibition is similar to that of the acceptor-side photoinhibition of PS II. The result that the stromal fraction containing a stromal protease(s) digests the cross-linked products of D1 also supports above view.

The mechanism of production of oxygen radicals under the moderate light and heat stresses is not known. Participation of lipid peroxidation is possible. Alternatively, some electron-transport component at the acceptor-side of PSII may be deteriorated under heat stress conditions, which generates reactive oxygen species to damage D1 and to inactivate PSII.

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