Supplementary Figures

Title: Superoxide and singlet oxygen produced within the thylakoid membranes both cause photosystem I photoinhibition

Running title: The photoinhibition mechanism in Photosystem I

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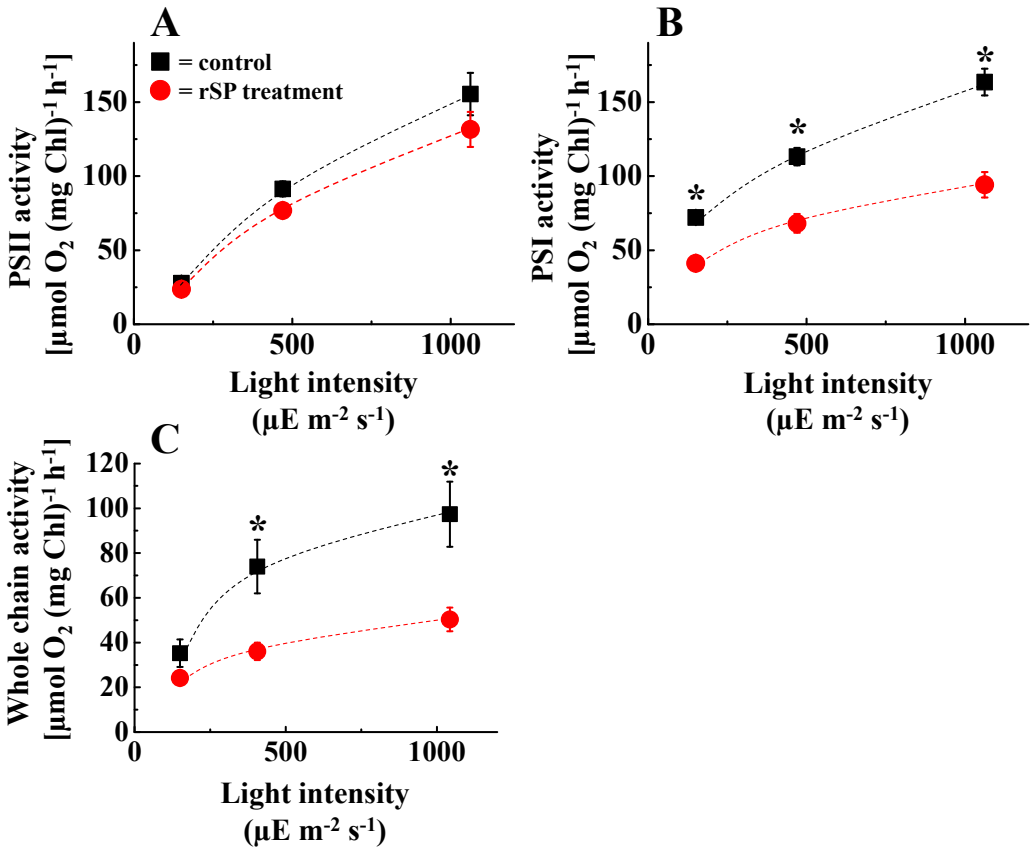
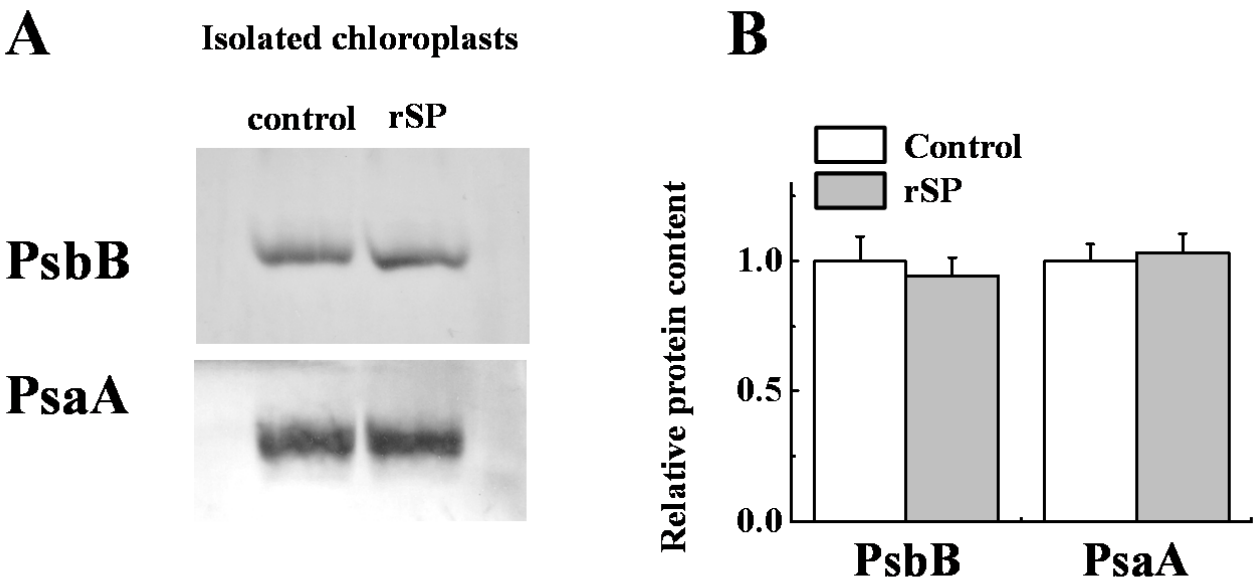


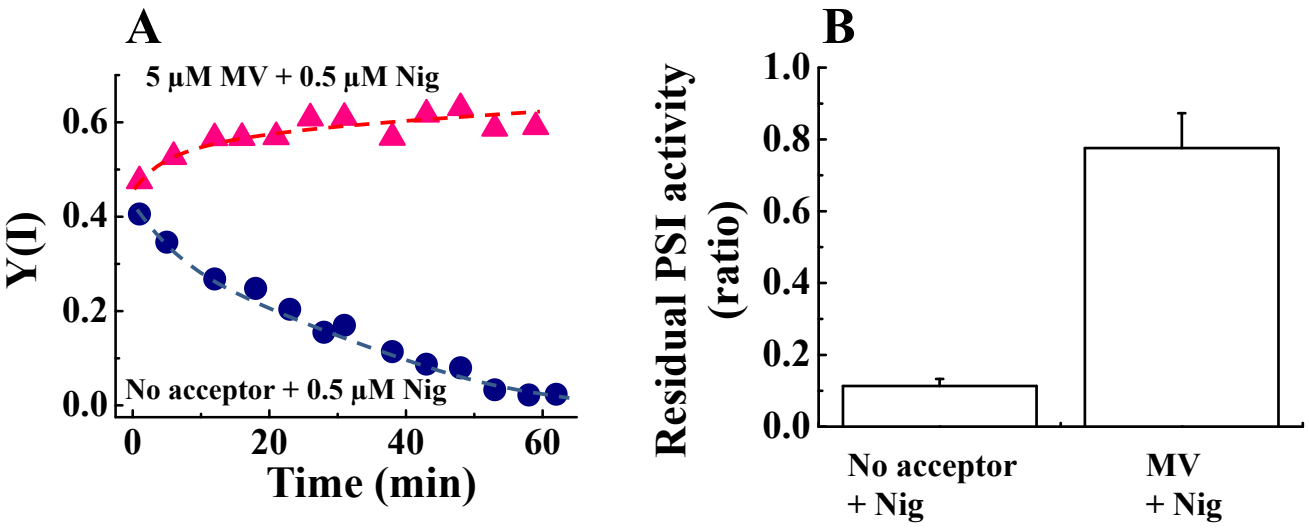
Supplemental Figure S1

The light response of PSII (A), PSI (B), and whole-chain photosynthetic electron transport activity (C) in isolated chloroplasts. The reaction mixture contained 30 µg ml^{-1} isolated chloroplasts, and the reaction mixture was maintained at 25°C. Photosynthetic electron activities were measured using an O_2 electrode (see Materials and Methods). Black square indicates photosynthetic electron activities of the control sample that was kept in the dark for 1 h. Red circle indicates photosynthetic electron activities of samples that were processed by SP treatment for 1 h. Data are expressed as the mean ± SEM of three independent experiments. Asterisks indicate a significant difference between the control sample and the rSP treatment sample (Student's *t*-test, $p < 0.05$).



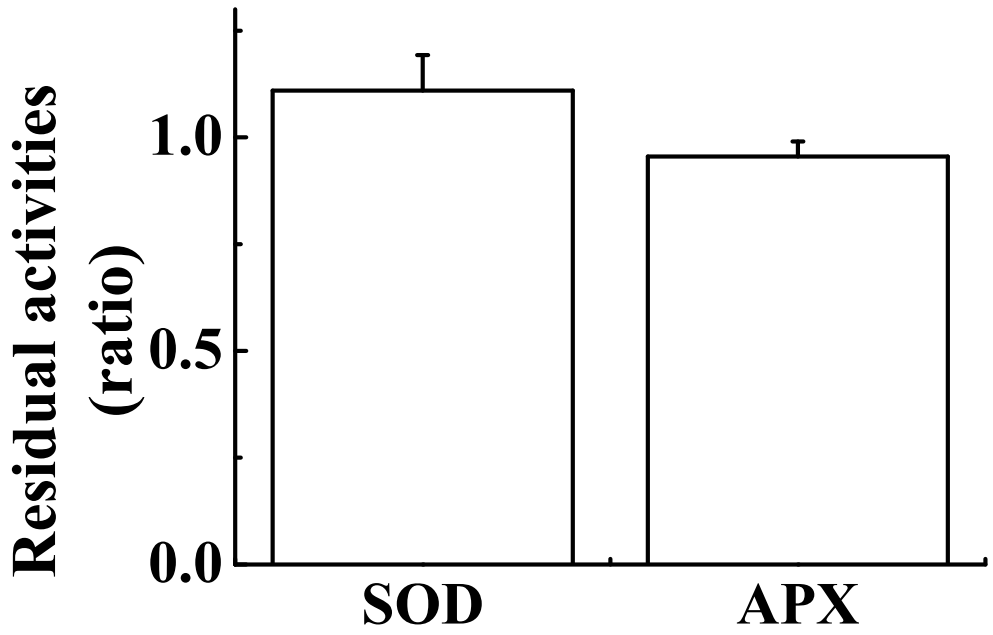
Supplemental Figure S2

The comparison of PSII and PSI core protein content in isolated chloroplast between before and after SP treatment. (A) Isolated chloroplasts before rSP treatment were used as a control, and isolated chloroplasts treated rSP treatment for 1h were used as an rSP treated sample. We used antiserum specific to PsbB and PsaA for quantifying PSII and PSI core protein content. The protein corresponding to 0.6 μg chlorophyll was loaded in each lane. The relative protein content is quantified (B). The protein content in control sample was set to 1. White bars indicate the protein content in control sample, and gray bars indicate the protein content in rSP treated sample. Data are expressed as the mean ± SEM of three independent experiments.



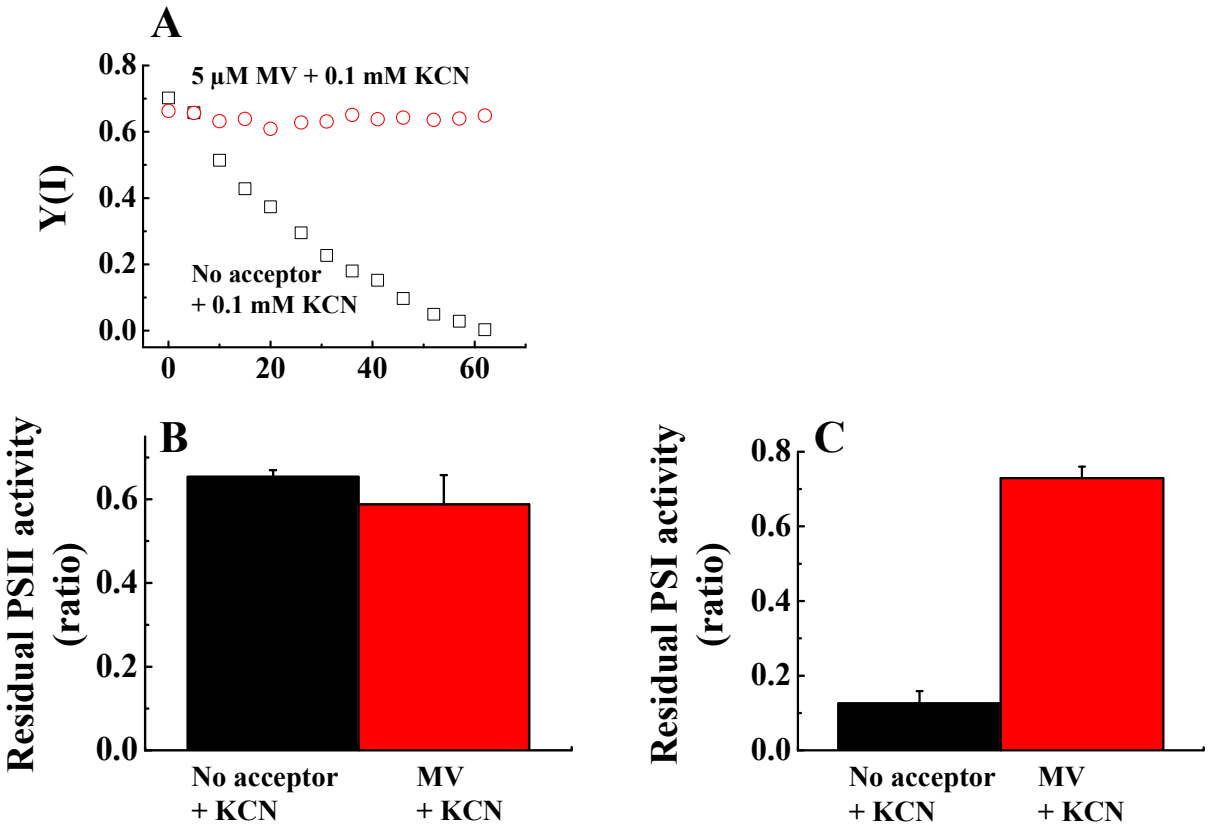
Supplemental Figure S3

The effect of nigericin on rSP treatment in the absence and presence of MV. The reaction mixture contains 30 µg ml^{-1} isolated chloroplasts and 0.5 µM nigericin, and the reaction mixture was maintained at 25°C. (A) The time-course analysis of Y(I) in isolated chloroplasts in the absence and presence of MV (5 µM). Experiments were repeated at least three times and representative data are shown. (B) The residual activity of PSI after rSP treatment in the absence and presence of MV. After rSP treatment, the reaction mixture was kept in the dark for 30 min and the Pm was measured. Data were normalized to the Pm before rSP treatment, and the data represents the residual activity of PSI after rSP treatment. Data are expressed as mean ± SEM of three independent experiments.



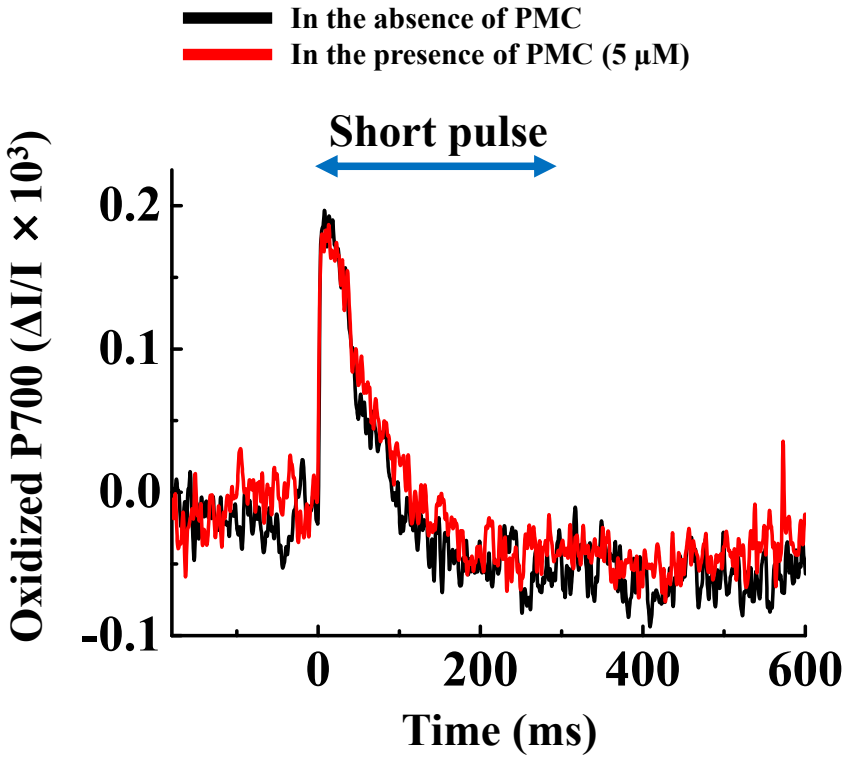
Supplemental Figure S4

The change in SOD and APX activities before and after rSP treatment. Chloroplastic SOD and APX activities were compared before and after rSP treatment and the residual activities are their ratio. Data are expressed as mean ± SEM of at least nine independent experiments. Absolute SOD activities were 233 ± 49 mU $(mg\ Chl)^{-1}$, and absolute APX activities were 5.6 ± 0.7 µmol $(mg\ Chl)^{-1}\ min^{-1}$.



Supplemental Figure S5

The effect of KCN on rSP treatment in the absence and presence of MV. The reaction mixture contains 30 μg ml^{-1} isolated chloroplasts and 0.1 mM nigericin, and the reaction mixture was maintained at 25°C. (A) The time-course analysis of Y(I) in isolated chloroplasts in the absence and presence of MV (5 μM). Experiments were repeated at least three times and representative data are shown. (B) The residual activity of PSII after rSP treatment in the absence and presence of MV. (C) The residual activity of PSI after rSP treatment in the absence and presence of MV. After rSP treatment, the reaction mixture was kept in the dark for 30 min and the Fv/Fm and Pm were measured. Data were normalized to the Fv/Fm and Pm before rSP treatment, and the data represents the residual activity of PSII and PSI after rSP treatment. Data are expressed as mean ± SEM of three independent experiments.



Supplemental Figure S6

The kinetics of oxidized P700 induced by a short-pulse in the absence of and in the presence of PMC (5 μM). The reaction mixture contained 30 μg ml^{-1} isolated chloroplasts, and the reaction mixture was maintained at 25°C. The data were obtained after rSP treatment was applied for 5 min. Black line shows the condition in the absence of PMC. Red line shows the condition in the presence of PMC. Experiments were at least three times. Short-pulse was illuminated every 10 s, and averaged data during three short-pulse illuminations were shown.