Mechanism of photoinhibition: magnetic field effect, singlet oxygen and kinetics

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Magnetic fields in the range of 100 mT are known to accelerate plant growth, but the phenomenon has lacked an explanation. We tested the hypothesis that magnetic fields lower the triplet yield of the recombination of the primary radical pair and thereby limit production of singlet oxygen ($^1O_2$). We found that magnetic fields really offer some protection against loss of PSII activity in high light, confirming that charge recombination mediates the formation of $^1O_2$. However, the magnetic field effect disappeared *in vitro* and also in the presence of lincomycin *in vivo*, indicating that $^1O_2$ exerts its effect on PSII by inhibiting PSII repair cycle, not by causing direct damage to PSII.

Triplet chlorophyll is also produced by intersystem crossing which is insensitive to moderate magnetic fields. To measure the importance of $^1O_2$ produced by types of chlorophyll triplets, we illuminated leaves of the Arabidopsis *vte1* mutant lacking α-tocopherol, an important $^1O_2$ scavenger. Leaves of the *vte1* mutant were found to be more susceptible to photoinhibition than the wild type in the absence but not in the presence of lincomycin. The result confirms the finding that $^1O_2$ is harmful to PSII mainly because $^1O_2$ inhibits repair of photoinhibited PSII.

Short flashes have been suggested to cause photoinhibition because $S_2Q_B^-$ charge recombination produces triplet chlorophyll. We tested the photoinhibitory efficiency of saturating single-turnover Xenon flashes *in vivo* and *in vitro* and found that the photoinhibitory efficiency of Xenon flashes is directly proportional to flash energy and independent of the time interval between the flashes. Because a saturating flash always causes the same number of recombination reactions irrespective of flash energy, the result indicates that $^1O_2$ produced due to $S_2Q_B^-$ recombination does not harm PSII. In fact, the quantum yield of photoinhibition was the same, whether Xenon flashes or continuous light was used, suggesting that flashes cause photoinhibition with the same mechanism as continuous light. Thus, photoinhibition is largely independent of PSII electron transport, which supports our earlier suggestion that the manganese ions of PSII have a role in photoinhibition.

Experiments with the *npq4* and *npq1* mutants of Arabidopsis demonstrated that non-photochemical quenching (NPQ) lowers the rate constant of photoinhibition by 25 %. We also tested whether the qI type of NPQ, associated with photoinhibited PSII centres, could protect the remaining active centres. Such protection would make the rate constant of photoinhibition to decrease with proceeding photoinhibition. However, photoinhibition did not deviate from first-order kinetics when leaves were illuminated for several hours in the presence of lincomycin. Also *in vitro*, photoinhibition strictly followed first-order kinetics until oxygen evolution was completely lost. Thus, qI does not offer any protection in addition to qE. Furthermore, persistent first-order kinetics indicates that the protective effect of NPQ must act on a separate, chlorophyll-dependent photoinhibition mechanism rather than acting on a second step of a manganese mechanism.