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Review

Photoinhibition of Photosystem II and photodamage of the oxygen evolving manganese cluster

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Contents

Abbreviations: $^1O_2$, singlet oxygen; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, dichlorophenol–indophenol; DPC, diphenylcarbazide; NPQ, non-photochemical quenching; OEC, oxygen-evolving complex; P_{680}, primary donor of PSII; PSII, Photosystem II; Q_A, quinone electron acceptor of PSII; ROS, reactive oxygen species; TyrZ, tyrosine-161 or D1 protein that functions as the immediate electron donor to P_{680}^+; UV, ultraviolet light (wavelength less than 400 nm); UVA, ultraviolet-A light (320–400 nm); UVB, ultraviolet-B (280–320 nm); UVC, ultraviolet-C (<280 nm).

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Abstract

Light drives photosynthesis, but at the same time, light also damages the photosynthesis machinery. The damaging reaction is termed photoinhibition, and its target is Photosystem II. The photodamaged Photosystem II reaction centre is repaired by degradation and resynthesis of the D1 protein. Both visible and ultraviolet light inhibit Photosystem II but ultraviolet light is much more efficient. This review describes the different hypotheses about the molecular mechanisms of photoinhibition. A special focus is put on the effect of light on the oxygen-evolving complex and on the recent hypothesis suggesting that light absorption of the manganese cluster of the oxygen-evolving complex plays a crucial role in photoinhibition. The manganese hypothesis is extended to show how the production of singlet oxygen by photoinhibited Photosystem II centres may occur.

Keywords: Action spectrum; Herbicides; Manganese; Non-photochemical quenching; Photoinhibition; Photosystem II; Singlet oxygen; Ultraviolet light

1. Introduction

It has long been understood that the photosynthesis machinery is sensitive to light and that continuous repair is needed to keep photosynthesis functional *in vivo* [1,2]. Studies of the mechanism behind the photosensitivity became one of the most popular topics in photosynthesis research after the observation of Kyle et al. [3] that the target of photoinhibition is Photosystem II (PSII) and that a 32-kDa membrane protein, later identified as the D1 protein of the PSII reaction centre [4], is selectively lost when isolated thylakoid membranes are subjected to intense light. This observation led to the concept of the photoinhibition-repair cycle that starts with photoinduced damage to the reaction centre complex of PSII and continues by subsequent enzymatic degradation and synthesis of the D1 protein [5–7]; for earlier reviews of photoinhibition, see [8–19].

Although the scheme of photoinhibition and repair in PSII (Fig. 1) is generally accepted and both the enzymatic degradation of the D1 protein [20–22] and mechanisms of D1 protein synthesis and subsequent activation of PSII [23–25] are known in detail, no consensus about the molecular mechanism(s) of the damage has been reached. The present review discusses the suggested mechanisms and compares experimental data with the predictions of the mechanisms. The role of the manganese cluster of the oxygen-evolving complex (OEC) is of special interest.

The term "photoinhibition" is used in the literature in various meanings. In this review, the word will be used to describe a reaction in which the electron transport activity of PSII is lost in such a manner that synthesis of the D1 protein is required before the activity returns. Reversible phases may exist in the pathway to an fully photoinhibited reaction centre, but the reversible lowering of the quantum yield of PSII photochemistry due to non-photochemical quenching of chlorophyll excitations [26,27] will not be included in the concept of photoinhibition. Furthermore, photoinhibition of Photosystem I (for review, see [28]) will not be discussed.

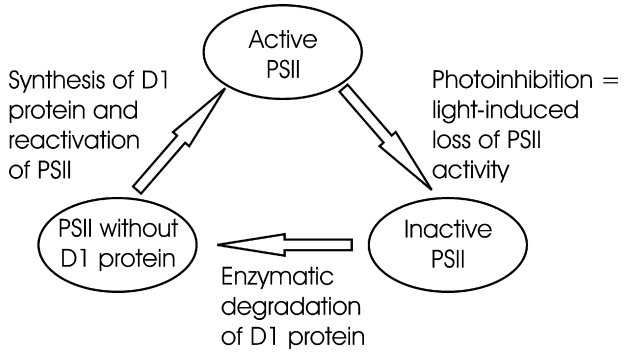

Fig. 1. Schematic representation of photoinhibition and repair of photoinhibited PSII.

2. Kinetics, quantum yield and action spectrum

The damaging reaction of photoinhibition is a simple first-order reaction [2,29,30]; deviations from first-order kinetics are seen only in severely photoinhibited leaves [31–33]. The most remarkable kinetic feature of photoinhibition is that the rate constant of the damaging reaction in isolated thylakoids [2], in intact leaves of higher plants [29,31,34–36] and in algal and cyanobacterial cells [37,38] is strictly proportional to light intensity. The direct proportionality has been shown from extremely dim light (photosynthetic photon flux density 6.5 μmol m^{-2} s^{-1}) to supersaturating light intensities [30] (Fig. 2). The rate constant of turnover of the D1 protein is also proportional to light intensity [39]. The quantum yield of photoinhibition, calculated on the basis of incident light, is in the order of 10^{-7} in both isolated thylakoids [40] and intact leaves of modest thickness [30]. The quantum yield was found to remain the same even when short Xenon flashes were used as photoinhibitory light [41,42]. The irradiance-independent quantum yield of photoinhibition contrasts with virtually all other light-induced reactions of the photosynthetic machinery. Light-intensity-independent quantum yield of photoinhibition can also be referred to as reciprocity between light dose and photoinhibition [43]. The term 'photon counter' [35] cannot be recommended because it gives an impression that photoinhibition is continuous wear-off instead of a probability phenomenon.

Reciprocity between light dose and photoinhibition holds only if experimental conditions, particularly those affecting the absorption properties of the experimental material, are carefully kept constant. Deviations from the reciprocity law have been found by measuring photoinhibition during the induction of photosynthesis [44] when the chloroplast metabolism undergoes rapid changes. Reciprocity is neither expected nor found when leaves are illuminated *in vivo* without treating them with an inhibitor of chloroplast translation [45,46] because in this

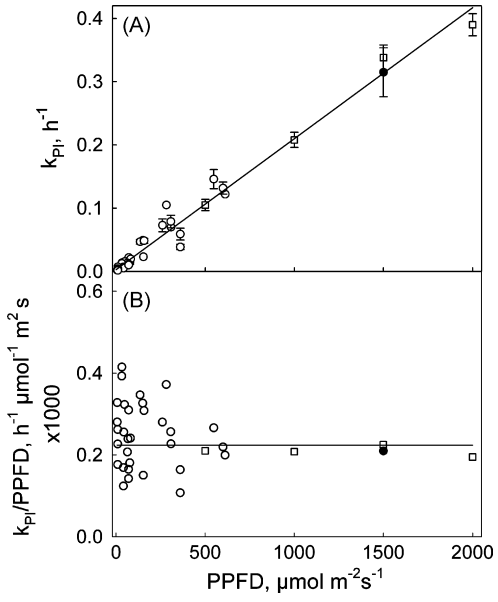

Fig. 2. (A) Dependence of the rate constant of photoinhibition (k_{PI}) on photon flux density. (B) The rate constant of photoinhibition divided by the photon flux density. Photoinhibition was measured by following the photoinhibitory decrease in the ratio of variable to maximum yield of chlorophyll *a* fluorescence in leaves of pumpkin plants grown in controlled conditions (open symbols) or in the field (closed symbol). Redrawn from [30].

case, balance between damage and repair determines the level of PSII activity.

The action spectrum of photoinhibition [2,37,42,47–52] shows highest values in UVC light, possibly with a peak at 250–260 nm and a low shoulder around 270–280 nm [2,47,48]. The photoinhibitory efficiency of light decreases smoothly throughout UVB (280–320 nm) and UVA (320–400 nm) regions towards longer wavelengths up to 450–500 nm. The visible-light tail of the spectrum above 450–500 nm is relatively flat, showing an overall efficiency of approximately 1% of the maximum value [2,42,49,51,52]. Most studies show a spectral peak in the red region between 650 and 700 nm, but the relative height of the red peak varies between different studies. High-precision measurements place the red peak at 672 nm in spinach and barley thylakoids [37]. In the action spectrum of photoinhibition of intact cyanobacterial cells, a low peak apparently reflecting the strong absorbance of phycobilisomes between 600 and 650 nm replaces the red peak [50].

3. What becomes inhibited in photoinhibition?

Activities of different parts of the PSII electron transfer chain are sequentially lost during photoinhibition. Several studies have identified OEC as the most sensitive component of isolated thylakoids under both visible [3,42,51,53] and ultraviolet (UV) light ([42,48,54–57]. Under both visible and UV light, the inhibition of oxygen evolution is associated with the release of approximately one Mn ion to the lumen [42,48]. The ability to reduce dichlorophenol–indophenol (DCPIP) in the presence of an artificial electron donor like diphenylcarbazide (DPC) is lost after the loss of oxygen evolution [3,42,51,53]. Furthermore, both optical [58] and EPR spectroscopy [59] have shown that inhibition of primary charge separation remains functional after the initial photoinhibition of oxygen evolution. When strong photoinhibitory illumination is applied under anaerobic conditions, an increase in the initial F_0 level of chlorophyll fluorescence is induced as an additional feature; the high F_0 level has been interpreted to show that the electron acceptor Q_A is converted to a stable reduced form [60,61]. Degradation of the D1 protein occurs after the inhibition of electron transfer activities of PSII [30,45,62–64]; for reviews, see [11,17]. The extrinsic proteins of OEC are released to the lumen concomitantly with degradation of the D1 protein [65].

4. Earlier hypotheses on the mechanism of photoinhibition

4.1. Acceptor-side and low-light hypotheses

In acceptor-side hypothesis [61,66], photoinhibition is assumed to start when the plastoquinone pool connecting the two photosystems is reduced under intense light. Experiments leading to the formulation of the acceptor-side hypothesis were done by illuminating PSII membranes (BBY particles) with strong light under anaerobic conditions [61]. In this condition, the Q_A electron acceptor of PSII stays mainly in the reduced form and the reduced state can be stabilized by protonation or double reduction. Illumination under anaerobic conditions was found to lead to inhibition of oxygen evolution and increase in the F_0 fluorescence value and both of these symptoms were found to be partially reversible with a time constant of tens of minutes [60,61,67,68]. The high F_0 level was interpreted to be associated with PSII centres with protonated Q_A^-, PSII centres with protonated Q_A^{2-}, and PSII centres missing the Q_A cofactor [61]. If the sample was saturated with oxygen after anaerobic illumination, reversibility was lost together with the loss of the EPR signal of the triplet state of the primary donor, suggesting that singlet oxygen ($^1O_2$) caused irreversible inactivation. The acceptor-side hypothesis has been extended to aerobic conditions where double reduced Q_A does not accumulate [69] by assuming that irreversible inhibition of PSII activity is caused by $^1O_2$ produced after stable reduction of Q_A. Photoinhibited thylakoids [70,71] and leaves [72,73] produce $^1O_2$ in the light and the rate of production depends on the degree of photoinhibition [72], but the reversibility associated with the $^1O_2$ producing stable reduced Q_A species has not been demonstrated under aerobic conditions.

Intense light is required for the formation of a stable reduced and protonated Q_A species, and the acceptor-side mechanism would have a lower quantum yield under dim light than under intense light [74]. A different mechanism, represented by the low-light hypothesis, was therefore needed to explain why photoinhibition also occurs under dim light. The low-light hypothesis is based on the fact that the quantum yields of the recombination reactions between the quinone acceptors of PSII and the S_2 and S_3 states of OEC increase with decreasing light intensity [75]. Recombination may lead to the triplet state of the primary donor, and the $^1O_2$ produced thereby might damage

PSII. The hypothesis was supported by results suggesting that the quantum yield of D1 protein degradation increases towards low light intensities [75]. However, conclusions about the light response curve of photoinhibition should be drawn with great care because the zero-order kinetic model used in the study [75] would exaggerate the quantum yield at low light, compared to high light. When the rate constant of photoinhibition is calculated by assuming first-order kinetics, a direct proportionality between the rate constant of photoinhibition and PPFD is obtained even under low light [30].

The main evidence for the low-light hypothesis is the observation that when short intense flashes are used as photoinhibitory light, the rate of photoinhibition depends on the length of the time interval between the flashes [75–78]. However, the photoinhibitory efficiency of Xenon flashes increases with flash intensity even though the flashes are saturating with regard to oxygen evolution [41,42], and increase in the rate of photoinhibition with light intensity has also been confirmed with laser pulses in our laboratory (M. Hakala, M. Keränen, T. Tyystjärvi, L. Khriachtchev, E. Tyystjärvi, unpublished data). These results contradict with the hypothesis that $^1O_2$ produced due to the $S_{2/3}Q_B^-$ recombination reactions is the source of damage in flash-induced photoinhibition, as the same number of recombination reactions is induced by a saturating pulse irrespective of how supersaturating the pulse is. The flash intensity dependence of flash-induced photoinhibition actually suggests that recombination reactions increase the concentration of the photoinhibition-susceptible state of PSII rather than directly cause the damage. Laser-pulse-induced photoinhibition is very slow in both Ca-depleted and Mn-depleted PSII [77], indicating that an active Mn complex is required for the inhibition reaction.

4.2. Donor-side hypothesis

4.2.1. Preparations with inhibited donor side

The donor-side photoinhibition mechanism is based on the observation that the residual electron transfer activity of PSII is rapidly lost during illumination of PSII complexes from which the oxygen-evolving Mn cluster was removed by NH_2OH or Tris treatment or by high-salt washing [79–88]. PSII becomes highly sensitive to light also if the advancement of the S-states is inhibited by Ca^{2+} or Cl^- depletion [88–90]. Donor-side photoinhibition can also be induced by treating thylakoids with cadmium [91] or copper [92].

In addition to the inhibition of the ability to reduce DCPIP in the presence of an exogenous electron donor, donor-side-inactivation has been shown to lead to loss of an EPR signal of oxidized tyrosine Z (TyrZ) [84,88], loss of a rapid component of the reduction of the oxidized primary donor (P_{680}^+) [40,84], and loss of photoactivation of OEC [79,82]. When PSII core complexes were illuminated in the absence of Ca^{2+} and Cl^-, a chlorophyll molecule was oxidized simultaneously with the inhibition of $CaCl_2$-dependent oxygen evolution [93]. The ChlD1 and ChlD2 molecules have the chlorine ring almost parallel with the membrane plane [94] like the chlorophyll first oxidized in donor-side photoinhibition [93], but stable oxidation of these chlorophylls is unlikely because both are part of P_{680}^+. The EPR signal related to oxidized tyrosine D (a tyrosyl residue of D2 protein) is lost later [84,88], and finally the capacity for primary charge separation and reduction of Q_A disappear [83,84]. Degradation of the D1 protein parallels the loss of the signal of oxidized tyrosine D [88].

The time-course of the inhibition of the residual electron transfer activity during donor-side photoinhibition is biphasic [40,82], suggesting that two different reactions function in donor-side photoinhibition. The partially contradictory observations showing that electron acceptors of PSII protect against [86], do not affect [40,87] or enhance donor-side photoinhibition [83] have also been interpreted to indicate the simultaneous operation of two reactions [86]. One reaction, prevailing under low light intensity, would start with generation of reactive oxygen species (ROS) on the reducing side of PSII, while the second reaction would be an oxidation of a vital component of the reaction centre by P_{680}^+ [86]. Inhibitors blocking reduction of Q_B offer protection against donor-side photoinhibition [80,87,89]. The involvement of a P_{680}^+ driven oxidation is supported by the observation that electron donors replacing the native Mn cluster, including DPC, Mn^{2+}, benzidine and NH_2OH, protect against donor-side photoinhibition [40,86,87]. The maximum quantum yield of the inactivation of electron transfer from TyrZ to P_{680} in Tris-washed thylakoids, obtained in low light, is around $0.5–1 \times 10^{-4}$ [40,81], which is three to four orders of magnitude higher than the quantum yield of photoinhibition in intact leaves [30].

The first results about the effect of oxygen on photoinhibition of donor-side-inhibited PSII suggested that oxygen is not involved in the reaction mechanism [85]. In later experiments [86], both anaerobic conditions and scavengers of oxygen radicals efficiently protected against photoinhibition of electron transfer from DPC to DCPIP in hydroxylamine-treated PSII membrane fragments. It has also been shown that donor-side-inhibited PSII produces reactive oxygen species, mainly H_2O_2, O_2^- and $^\bullet OH$, in the light [70,71]. Production of H_2O_2 in Cl^- depleted PSII may also explain why Cl^- depleted PSII membranes are more susceptible to photoinhibition than Ca^{2+} depleted ones [90,95].

4.2.2. Stochastic donor-side inhibition

Although the donor-side mechanism has been seen to function in pure form only in preparates with an inactive OEC, donor-side inhibition has been suggested to occur with a low efficiency *in vivo* [43,80]. In this hypothesis, OEC is assumed to be stochastically unable of reducing P_{680}^+. When rapid reduction does not occur, P_{680}^+ lives long enough to oxidize a wrong component of PSII. The PSII centres giving rise to the misses of the flash oxygen sequence [96–99] form an obvious piece of evidence supporting the presence of PSII centres in which the lifetime of P_{680}^+ is long.

The experimental light response curve of donor-side photoinhibition occurring after chemical inactivation of OEC shows light saturation [40,81,91], and the stochastic donor-side inhibition would also be saturated by light intensities that saturate photosynthesis [74].

4.3. Hypotheses relating photoinhibition to exogenously generated reactive oxygen species

Two research groups have suggested that photoinhibition might be mediated by $^1O_2$ produced outside the electron transport chain of PSII. In this context, photoinhibition comes near to the concept of Type II phototoxicity phenomena (for review, see [100]). Jung and Kim [49,101–103] suggested that the action spectrum of photoinhibition indicates generation of $^1O_2$ by iron-sulfur centres and cytochromes of thylakoids. The hypothesis is supported by data showing that if plants are grown with excess iron, the non-heme iron content of chloroplasts increases and blue and UVA-light-induced photoinhibition of PSII is enhanced [104]. Furthermore, photoinhibition of PSII core complexes is enhanced by adding isolated cytochrome b_6/f complexes to the reaction mixture [105]. PSI contains most of the iron-sulfur centres of thylakoids, but PSI does not produce $^1O_2$ [106], and addition of PSI preparates does not enhance PSII photoinhibition [105].

The hypothesis of Santabarbara et al. [37,107,108] also explains photoinhibition by the action of $^1O_2$ generated outside functional PSII. The authors found that the red peak of the action spectrum of photoinhibition is blue-shifted by 2–4 nm compared to the absorption spectrum of the antenna system of PSII. A similar blue-shift was found in the action spectrum measured from thylakoids of both wild type and the chlorophyll *b*-less *chlorina f2* mutant of barley [37]. The blue-shifted red peak of the action spectrum of photoinhibition resembles the excitation spectrum of chlorophyll phosphorescence, suggesting that formation of the triplet state of chlorophyll is involved [37]. The blue-shift, as well as the low efficiency of protection by quenching of chlorophyll excitations with dinitrobenzene or other artificial quenchers of the singlet excited state [109–111] led to the suggestion that photoinhibition both *in vivo* and *in vitro* is caused by $^1O_2$ produced after formation of triplet states of weakly coupled chlorophylls. Such chlorophylls could be present in damaged pigment-protein complexes and in intermediate states of the biosynthesis of pigment-protein complexes.

4.4. Ultraviolet light—mechanisms common with visible-light photoinhibition and mechanisms specific to UV light

4.4.1. Inhibition of photosynthesis by UV light

In addition to the general cytotoxic effects of UVB and UVC (<280 nm) wavelengths (for review, see [112]), UV light causes multiple effects on the photosynthesis machinery, including loss of plastoquinone [55,113–115], Rubisco [116] and chlorophyll [116], and degradation of phycobiliproteins in cyanobacteria [117]. Electron transport through PSII is highly sensitive to UV light [2,42,46,48,49,51,52,54,113,114,118,119], and the inhibition of electron transport through PSII under UV light is accompanied by lowering of variable chlorophyll *a* fluorescence [46,116,120–122] and lowering of the intensities of the thermoluminescence Q and B bands [122,123]. The different parts of the PSII electron transfer chain become inhibited at very different rates, and it is not clear which damaging reactions occur independently and which should be classified as secondary reactions. Two phases will be distinguished here, the rapid inhibition of OEC and the slower damaging reactions affecting both acceptor and donor side of PSII.

The other photosystem, PSI, is not affected by UVB [54,121,124] or UVA light [122] and is relatively insensitive to UVC light [125]. PSI also tolerates high-intensity visible light [126]. Thus, the difference in photosensitivity between the two photosystems extends from UV to visible light, suggesting the existence of a mechanism that damages PSII under both UV and visible light.

The activity of UV-photoinhibited PSII is restored *in vivo* via a similar repair process that restores the activity of PSII after visible-light photoinhibition [114,127]. The D1-synthesis-dependent repair of PSII occurs concomitantly with UV-light-induced damage [57,118,119,128–133]. Low temperature exacerbates UV photoinhibition *in vivo* [134] apparently by slowing down the concomitant recovery, and the herbicide 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) slows down D1 protein degradation also under UVB light [135]. The action spectrum of the repair of UV-photoinhibited PSII indicates dependence on photosynthesis [128], reflecting the dependence of the synthesis of D1 protein on functional photosynthesis [136,137]. In cyanobacteria, both UV and visible-light-induced photoinhibition cause an increase in the transcription of the *psbA* genes encoding the D1 protein [50,138,139]. Furthermore, acclimation to UV light causes similar metabolic changes in photosynthetic organisms as acclimation to high-intensity light, like faster repair of photoinhibited PSII [117] and switching to a high-light specific form of the D1 protein in cyanobacteria that have two forms of the D1 protein [132,133].

Experimental evidence about the fragmentation pattern of the D1 protein during illumination of photosynthetic material with UV light is contradictory, suggesting either similar fragmentation under visible and UV light [125,140] or different fragmentation patterns [55,141]. The generation of a 20-kDa C-terminal D1 protein fragment under UVB light occurred at 0 °C, suggesting that no protease is required [142]. Cleavage of the D1 protein to specific fragments under UVB illumination occurs only in preparations that contain a functional manganese complex [55], and protein degradation in PSII reaction centre complexes in UVB light is independent of the presence of the quinone acceptors [143], suggesting that protein degradation is triggered by inhibition of OEC activity, not by the damage on the acceptor side of PSII. The D2 protein is also degraded under UV light [122,125,135,143] with a slower rate than the D1 protein.

4.4.2. Fast phase: inhibition of OEC in UV light

The fastest phase of UV-induced photoinhibition of PSII is inhibition of the activity of OEC, as evidenced by the observation that after inhibition with UV light, the rate of electron transport through PSII can be partially restored with DPC [42,48,54–57]. Two reports show no restoration after treatment with UVB [54,114], and in spinach thylakoids, DPC could not restore the UVB-induced loss of the 320-nm absorption change related to oxidation of Q_A [121]. Furthermore, the inhibitory efficiency of 308-nm UVB laser flashes was found to depend on the S-

state [144]. The conclusion that OEC is the primary target of UV light in PSII is supported by the observation that the inhibition of the residual electron transport activity in Mn-less PSII is much slower in UVB [55] and UVC light [42] than inhibition of oxygen evolution in intact thylakoids. The preferential inhibition of OEC activity is not a UV-specific feature, as photoinhibition begins with the loss of OEC activity under visible light, too [3,42,51,53].

The molecular mechanism of inhibition of OEC in UV light is not known in detail. Both thermoluminescence [123] and 830-nm absorbance measurements [48,145] indicate that UVB light causes full inhibition of the S-state cycle instead of blocking the cycle at any specific state. A S_1-state-specific increase in a 90-μs component of P_{680}^+ reduction was interpreted to indicate either a specific UVB effect on S_1 state or an effect on the dark relaxation of OEC to S_1 [145]. Release of slightly more than one Mn ion per one inhibited PSII unit occurs during photoinhibition of thylakoids under UVB [48] and UVC light [42]; three Mn per inhibited PSII were found to be released under strong UVA light [57]. UVB illumination also causes release of Mn from PSII membrane particles [146]. These data suggest that loss of the activity of OEC is associated with the release of Mn to the lumen.

The photoreceptor of the UV-induced damage to OEC is apparently the Mn complex itself. The photoinhibitory efficiency starts to increase towards shorter wavelengths at 450–500 nm and the efficiency of UVA light is very high compared to visible light (Fig. 3). These spectral features are common to synthetic Mn(III) and Mn(IV) containing models of the Mn cluster [147–149] and also to the Mn catalase enzyme [150]. The absorption spectra of Mn complexes depend strongly on the ligands, and therefore an exact match between the action spectrum of photoinhibition and the absorption spectrum of any of the model complexes is neither expected nor found.

An excited state of an Mn ion would probably be too short-lived for a chemical reaction like Mn release to take place, and therefore the Mn-sensitized inhibition of OEC probably involves a light-induced rapid conformational change of the Mn cluster. One possibility is that a transient spin crossover occurs due to excitation. Spin crossover phenomena are known from Fe(II) and Mn(II) and Mn(III) coordination compounds that have alternative spin configurations [151–153], and absorption of light can induce the crossover phenomenon [151].

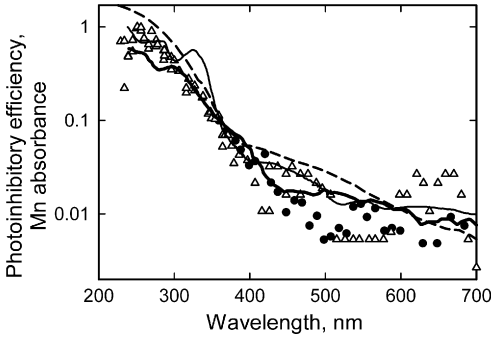

Fig. 3. Comparison of action spectra of photoinhibition (experimental points; [2], triangles; [49], circles) with absorption spectra of three model complexes of the oxygen-evolving Mn cluster, (Mn(III)gluconate i 0.01 M gluconate ([147], dashed line); Mn(III)–O–Mn(IV)–(*N*,*N*-bis(2-pyridylmethyl)-*N*′-salicyliden-1,2-diaminoethane)$_2$ ([148], thick solid line); [Mn_2(III,IV)(μ-O)$_2$(2,2′:6,2″-terpyridine)$_2$(CF_3CO_2)$_2$]$^+$) ([149], thin solid line). The spectra are normalized so that the value at 360 nm is 0.1. Redrawn from [42].

Inhibition of OEC activity by UV light leads to an increase in slow (μs) components of reduction of P_{680}^+ [48,145]. Slow reduction of P_{680}^+ increases the probability of recombination of the $P_{680}^+Q_A^-$ charge pair with 100–200 μs kinetics [154,155]; the $P_{680}^+Q_A^-$ recombination also leads to 'misses' in the oxygen sequence [97]. The rapid $P_{680}^+Q_A^-$ recombination is not seen in chlorophyll *a* fluorescence data because both P_{680}^+ and Q_A cause quenching of fluorescence, but a slower recombination between $TyrZ^+$ and Q_A^- can be seen as decrease in fluorescence yield in the presence of DCMU [130]. The $TyrZ^+Q_A^-$ recombination has components with half-times in the 800–900 μs and 8–10 ms range [130].

4.4.3. Slow phase: UV-induced damage to the rest of PSII

UV light excites the chlorophylls to some extent, and therefore the same mechanisms that inhibit the residual electron transfer activity in OEC-less PSII also function with low rate when the OEC activity has been inhibited by UV light. However, the slow phase of UV photoinhibition consists mainly of UV-specific damaging reactions. The slow phases of the UV-induced inhibition of PSII include loss of the EPR signals of the $Q_A^-Fe^{2+}$ acceptor complex and oxidized tyrosine D [146], loss of atrazine binding sites [48], decrease in the DCMU sensitivity of the Q_B binding site [115,123], and slowing down of electron transfer from Q_A^- to Q_B [130]. Degradation of the D1 protein and the loss of the EPR signals of oxidized tyrosine D the $Q_A^-Fe^{2+}$ acceptor complex actually occur with the same rate under UVB illumination [146]. Interestingly, loss of the EPR signal of tyrosine D parallels the degradation of the D1 protein also during visible-light photoinhibition [88].

The photoreceptors of the slow phases of UV damage are not known, but the electron acceptor quinones, aromatic amino acid residues and the oxidized tyrosyl radicals $TyrZ^+$ and $TyrD^+$ are likely candidates.

4.4.4. UV-photoinhibition and oxygen

The role of oxygen in UV-induced photoinhibition is not clear. Both superoxide and $^1O_2$ are produced during photoinhibition under UV and visible light, UV light inducing mainly production of superoxide while visible-light-photoinhibition preferentially induces production of $^1O_2$ [70–73,156,157]. UV-induced photoinhibition occurs in the absence of oxygen [42,158].

5. Photoinhibition mechanisms and effects of environmental and physiological factors

5.1. Oxygen

5.1.1. Photoinhibition under aerobic and anaerobic conditions

Illumination leads to inhibition of PSII activity under anaerobic conditions both *in vivo* and *in vitro* [42,158–164], but

anaerobic photoinhibition is partially reversible [60,61,67,68]. The reversion occurs only under aerobic conditions [68], but photoinhibition induced under aerobic conditions is not reversible [165]. Anaerobicity inhibits D1 degradation in oxygen-evolving PSII particles [162,166] but D1 degradation proceeds rapidly in Tris-washed membranes even under anaerobic conditions [85].

The occurrence of photoinhibition under anaerobic conditions suggests that either a specific photoinhibition mechanism functions under aerobic conditions, or that the reaction mechanism of photoinhibition is independent of oxygen. The similarity of the action spectra of aerobic and anaerobic photoinhibition [42] suggests that the mechanism of anaerobic photoinhibition is the same as that of aerobic photoinhibition. Even if we assume that the mechanism of photoinhibition does not need oxygen directly, the presence of oxygen may affect the rate of photoinhibition by affecting the state of PSII via redox equilibria. In anaerobic conditions, ambient redox potential has a strong effect on the rate of photoinhibition in both active PSII [167] and in Tris-washed PSII membranes [168].

5.1.2. Effect of ROS on photoinhibition and D1 protein turnover

ROS can damage PSII [169,170] and cause cleavage of the D1 protein to specific fragments [166,171–173], and scavengers of ROS slow down photoinhibition *in vitro* [53,64,174]. Chemical quenchers of ROS have also been shown to help the chloroplast to maintain the activity of PSII *in vivo* [175–177]. However, experiments done in the presence of the concurrent repair of photoinhibitory damage [175–177] cannot distinguish between effects on the damaging reaction and effects on the repair of photoinhibited PSII, and separate experiments show that $^1O_2$ does not enhance the damaging reaction if photoinhibition *in vivo* is measured in the presence of a protein synthesis inhibitor [178,179]. Synthesis of the D1 protein is inhibited by $^1O_2$ [19,178–180], which may explain why inhibition of the synthesis of the $^1O_2$ quencher α-tocopherol leads to rapid loss of PSII activity and D1 protein in *Chlamydomonas* [176,181] and to photosensitivity in *Arabidopsis* [182]. An α-tocopherol deficient mutant of *Synechocystis* sp. PCC 6803 was not particularly sensitive to photoinhibition [183]. The localization of the main effect of $^1O_2$ on protein synthesis is also supported by the observation that recovery from photoinhibition was retarded in α-tocopherol deficient tobacco plants [184]. These data suggest that the target of the protective effect of α-tocopherol is the protein synthesis machinery rather than PSII itself.

A possible reason for the apparent effect of reactive oxygen species *in vitro* and the contradictory observations *in vivo* is that the ROS effects are strongly concentration dependent, and protein synthesis is the most ROS-sensitive function of the chloroplast. ROS scavengers of chloroplasts and cyanobacteria do not let the *in vivo* concentrations of ROS grow high enough to significantly affect PSII. The observation that overproduction of chloroplast-targeted superoxide dismutase did not protect against the damaging reaction of photoinhibition *in vivo* [185] may also suggest that the activity of this antioxidant enzyme is high enough for the plant to cope with high light. Under low temperature where the antioxidant enzymes function slowly, the damaging reaction of photoinhibition was slowed down by over-expression of the stromal antioxidant enzymes superoxide dismutase, ascorbate peroxidase and glutathione reductase [186].

5.2. Electron flow through PSII

5.2.1. The relationship between photoinhibition hypotheses and PSII excitation pressure

In the early days of photoinhibition research, it was often assumed that the rate of photoinhibition is determined by excitation energy absorbed in excess of the use of excitation energy in photosynthesis. A mathematical model of this assumption divided absorbed energy to fluxes to photochemistry, thermal dissipation and excess energy [187]. Experimental evidence supporting the excess energy hypothesis was obtained from photoinhibition of *Chenopodium* grown with different nitrogen levels [188] but when the photochemical usage of excitation energy was deliberately lowered by blocking the Calvin-Benson cycle, the results did not support the excess energy hypothesis [42].

A major problem of the excess energy hypothesis is that virtually all unused excitation energy ends up as heat or fluorescence, and the actual photoinhibitory energy flow is in the range of 10^{-7} times incident photon flux density. Because this number would hardly qualify as a definition of excess energy, the excess energy analysis has to define what percentage of heat dissipation is harmless and what percentage is potentially harmful. So far, the only excess energy parameter "E" defined in this way [187] has been shown to be physically wrong [189]. The "E" parameter was calculated by summing the energy flow to photochemistry (proportional to Φ_{PSII}) and a potential dissipative energy flow (proportional to $1 - F'_V/F'_M$). The critique is based on the fact that Φ_{PSII} is measured in the light whereas the potential dissipative energy flow is meaningful only if all PSII centres are open and the rate constant of photochemistry has its dark-adapted value [189].

Rates of both reduction of Q_A and oxidation of Q_A^- affect the steady-state proportion of closed PSII centres, sometimes called "PSII excitation pressure". The effect of PSII excitation pressure is crucial for testing the importance of the acceptor-side mechanism because the rate of the formation of the stable reduced forms of Q_A [61] would be directly proportional to the concentration of normal, singly reduced Q_A^-. Therefore all factors that close reaction centres would also speed up acceptor-side photoinhibition. The donor-side [43] and singlet oxygen hypotheses [49,107] would be less affected. Blocking electron transfer from Q_A^- to Q_B would slow down damage caused by long-lived P_{680}^+ in donor-side photoinhibition because the recombination reaction $P_{680}^+Q_A^- \rightarrow P_{680}Q_A$ would deplete the pool of long-lived P_{680}^+. Mechanisms based on $^1O_2$ production outside functional PSII, in turn, would be independent of electron transport through PSII. The relationship between different photoinhibition mechanisms and electron transport via PSII will be discussed below in more detail.

5.2.2. Effects of PSII herbicides

A classical way to block electron transport from Q_A^- to Q_B is treatment with a herbicide like DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (see [190]). All herbicides that bind to the Q_B site in PSII increase the lifetime of Q_A^- by approximately three orders of magnitude, which is expected to cause a radical increase in the rate of acceptor-side photoinhibition. However, the effect depends on the herbicide. DCMU protects against photoinhibition of oxygen evolution and slows down D1 protein degradation in higher plants, algae and cyanobacteria [175,191–194]. DCMU also slows down photoinhibition of oxygen evolution in spinach thylakoids [195] and in PSII membranes [196]; in some cases no effect or even slight enhancement of photoinhibition was seen [166,198]. In contrast to DCMU, phenolic PSII herbicides like 2-bromo-3-methyl-6-isopropyl-4-nitrophenol (BNT), ioxinyl and dinoseb speed up photoinhibition both *in vivo* and *in vitro* [194,196,198]. Like PSII herbicides, also formate blocks electron transfer from Q_A^- to Q_B; the inhibition occurs because formate replaces a bicarbonate ion bound to the acceptor side of PSII. Formate has a slight protective effect against photoinhibition of thylakoids in aerobic conditions [199].

The protective effects of DCMU and formate might be explainable within the framework of acceptor-side photoinhibition by assuming that the formation of the protonated or double reduced state of Q_A requires a protein conformational change which is precluded by the binding of DCMU to the Q_B site [195] or by binding of formate in place of bicarbonate. Binding of a phenol-type herbicide might enhance the required conformational change. However, the urea type herbicides like DCMU that partially protect against photoinhibition also increase the redox potential of the Q_A/Q_A^- pair [200], whereas the phenolic PSII inhibitors that enhance photoinhibition, lower the redox potential of the Q_A/Q_A^- pair [200]. These data suggest that the recombination reaction $Q_A^- \rightarrow Q_A$ affects the rate of photoinhibition [200]. Faster photoinhibition in the *Thermosynechococcus* D2 protein mutant A249S in which the redox potential of the Q_A/Q_A^- pair is lowered, confirms this conclusion [201]. The rationale is that if the redox potential of the Q_A/Q_A^- pair is lowered from the wild-type value, then the recombination reactions involving Q_A^- more probably follow the route that may produce $^3P_{680}$ and lead to production of $^1O_2$.

The effect of the redox potential of the Q_A/Q_A^- pair calls for a photoinhibition mechanism in which recombination reactions involving Q_A^- have a role. The low-light mechanism [76] would be an apparent alternative but the $S_2Q_A^-$ recombination saturates at very low light [76] and this reaction would therefore not explain why the rate of photoinhibition increases with light intensity. Furthermore, recombination reactions have no role in the stochastic donor-side mechanism [43], and the redox midpoint potential of the Q_A/Q_A^- pair would not affect the rate of photoinhibition in the acceptor-side hypothesis [61] because stable reduced Q_A species, by definition, do not participate in recombination reactions. It should be kept in mind that the effect of the redox potential of the Q_A/Q_A^- pair on the rate of photoinhibition is clearly observable but minor.

5.2.3. Effect of blocking electron flow after PSII

Instead of using chemicals that affect PSII, oxidation of Q_A^- can be slowed down by mutations and chemicals that do not directly affect PSII. Reduction of the amount of functional PSI has often led to a highly photosensitive phenotype. A PSI-less mutant of the cyanobacterium *Synechocystis* sp. PCC 6803 grows only under low light intensities [202], and the F8 mutant of *Chlamydomonas* [203,204] and PSI-D-antisense *Arabidopsis* plants [205], both with a greatly reduced amount of functional PSI, are more sensitive to high light than the respective wild-type organisms. Tobacco plants containing an antisense construct against the ferredoxin-NADP(H) reductase showed symptoms of light-dependent photo-oxidative damage [206]. No one of these mutants was actually tested for the damaging reaction of photoinhibition, and the reason for their photosensitivity may therefore be related to the repair of photoinhibited PSII. Photoinhibitory loss of variable fluorescence and degradation of the D1 protein occurred more slowly in the PSI-less B4 mutant of the green alga *Chlamydomonas* than in the wild type [207], indicating that the absence of functional PSI does not universally speed up photoinhibition.

Varying the activity of the Calvin-Benson cycle is another way to test the effect of PSII excitation pressure on photoinhibition *in vivo*. Treatment of pea leaves with D,L-glyceraldehyde, an inhibitor of the Calvin-Benson cycle, led to complete inhibition of photosynthesis and an almost complete reduction of Q_A in the light, but photoinhibition of oxygen evolution was slightly slower in D,L-glyceraldehyde-treated leaves than in control leaves when lincomycin was used to block D1 protein synthesis [42]. Neither did chemical inhibition of the Calvin-Benson cycle affect the rate of photoinhibition in *Chlamydomonas* cells [136]. Mutations affecting the activity of the Calvin-Benson cycle have led to a variety of responses with regard to photosensitivity. A spontaneous mutation in the large subunit of Rubisco in *Oenothera* lowered the rate of photosynthesis, increased the proportion of closed PSII reaction centres and led to photosensitivity of the intact plants [208]. A tobacco mutant lacking the large subunit of Rubisco [209] shows a lower steady-state level of oxygen evolution under high-light illumination, compared to wild-type plants, and the synthesis rate of the D1 protein was slower in the Rubisco-less plants compared to the wild type. However, the 10-6C mutant of *Chlamydomonas* with a missense mutation in the Rubisco large subunit shows deficiency in the repair of photoinhibitory damage, but the damaging reaction of photoinhibition has the same rate in the 10-6C mutant as in the wild type [136]. Furthermore, plants transformed with an antisense construct of the small subunit of Rubisco may show strong reduction of photosynthetic electron transport but can be propagated under normal greenhouse conditions [210,211]. Similarly, antisense silencing of glyceraldehyde-3-phosphate dehydrogenase led to reduced rate of photosynthesis, but the plants did not show symptoms of chronic photoinhibition [212]. Importantly, results showing photosensitivity [208,209] were obtained in the presence of the repair cycle of PSII, while direct measurements of the damaging reaction of photoinhibition failed to show an effect of the rate of the Calvin-Benson cycle [42,136]. The results indicate that PSII photoinhibition is not enhanced if

PSII excitation pressure is increased by lowering the rate of the Calvin-Benson cycle.

Chilling temperature slows down the Calvin-Benson cycle, and therefore PSII excitation pressure increases if temperature is lowered but light intensity is kept constant. Symptoms of photoinhibition appear soon when intact plants or cyanobacteria are exposed to light at temperatures substantially below their growth optimum [12,13,134,213–215], and a constant ratio has been obtained between photochemical quenching and photoinhibition in rye leaves at different temperatures [216]. However, the difference in the rate of photoinhibition induced at growth temperature or low temperature disappears in the presence of a chloroplast protein synthesis inhibitor, indicating that the enhancement of photoinhibition by low temperature is purely due to slowing down of the concurrent recovery at low temperature [38,217]. Furthermore, unsaturation of fatty acids of membrane lipids which facilitates low-temperature photosynthesis, improves the ability of the plant to repair photoinhibited PSII at low temperature but has no effect on the damaging reaction [218]. Low temperature actually slows down photoinhibition in isolated thylakoids [197].

The availability of PSII electron acceptors can also be limited by illuminating intact leaves in the absence of CO_2. In this case, the damaging reaction of photoinhibition was faster in *Lemna* in CO_2-free air or in pure nitrogen atmosphere than in the presence of CO_2 [164].

Reduced or missing activity of the cytochrome b_6/f complex slows down the oxidation of Q_A^- but does not seem to enhance photoinhibition. A mutant of the higher plant *Lemna* lacking a functional cytochrome b_6/f complex [219,220] has not been characterized as particularly photosensitive, and strong reduction of photosynthesis by antisense silencing of the cytochrome b_6/f complex in tobacco did not lead to notable photosensitivity [221]. Loss of variable fluorescence and degradation of the D1 protein under high light occurred more slowly in the cytochrome b_6/f-less mutant D6 of the green alga *Chlamydomonas* than in the wild type [207,222]. Photoinhibition was slow also in the plastocyanin-deficient AC208 mutant of *Chlamydomonas* [207]. The *immutans* mutant of *Arabidopsis* in which the plastid terminal oxidase does not function, is slightly more sensitive to photoinhibition than the wild type [223]. These data do not directly test the effects of the mutations or antisense constructs on the damaging reaction of photoinhibition, as the concomitant recovery from photoinhibition was not blocked during illumination. Transgenic tobacco plants with lowered rate of photorespiration were photosensitive [224], which was thought to indicate that photorespiration acts as a valve that lowers PSII excitation pressure. However, reduction of photorespiration was recently shown to impair the repair of photoinhibited PSII and to have no effect on the damaging reaction of photoinhibition [225].

The great variation in effects of treatments that affect PSII excitation pressure indicate that PSII excitation pressure does not universally determine the rate of photoinhibition. Side effects rather than effects on the electron transport rate may often cause the differences in photosensitivity obtained with chemical treatments and mutations. Most side effects lower the rate of repair of photoinhibitory damage, but the damaging reaction can be affected, too. The most important class of side effects modifying the rate of the damaging reaction is effect on leaf chlorophyll content. For example, treatment of bean plants with copper caused an increase in the quantum yield of photoinhibition [226] but this increase was fully explained by the lower chlorophyll content of copper-stressed bean plants [227].

5.3. Antenna size and quenching of chlorophyll excitations

Both the size and light harvesting efficiency of PSII antenna are regulated as a function of the light intensity in higher plants (for reviews, see [15,228,229]), and manipulation of antenna size and non-photochemical quenching (NPQ) may give insight into the photoreceptors of photoinhibition. Most [35,45,230,231] although not all [46] *in vivo* studies show that the small antenna size of plants grown under high light offers little or no protection against photoinhibition. Furthermore, the rate of *in vitro* photoinhibition of oxygen evolution is the same in PSII preparations with different antenna sizes [197]. Photoinhibition of reduction of Q_A occurred slightly more slowly in thylakoids of the Chl *b* deficient *chlorina f2* mutant of barley than in wild-type thylakoids [232], and PSIIα that has a larger antenna size was more rapidly photoinhibited than PSIIβ [233]. The latter result may, however, not indicate antenna size dependency, as PSIIβ may be considered to be an intermediate of the repair cycle of PSII [11].

The effect of non-photochemical quenching of chlorophyll excitations has been studied both by inducing the quenching chemically and by studying mutants deficient in natural NPQ. *In vitro* experiments with *m*-dinitrobenzene and quinones as quenchers [37,109–111] showed only 20–30% maximum protection.

The *Arabidopsis* mutants *npq4* [234] and *psbs* [235], both lacking a functional PsbS protein, as well as mutants like *npq1* [234] that lack a functional violaxanthin de-epoxidase, have been extremely important for understanding the relationship between photoinhibition and NPQ. The PsbS-deficient mutants have very little NPQ, and the NPQ level of the *npq1* mutant is intermediate between *npq4* and the wild type [234]. NPQ-deficient mutant plants can be propagated in normal greenhouse conditions, but low-light-grown NPQ-deficient mutants show more severe photoinhibition upon transfer to high light than the wild type [236]. The *npq4* and *npq1* mutants also show lower seed production in field conditions than the wild type of *Arabidopsis* [237]. Furthermore, over-expression of the PsbS protein improves the high-light tolerance of *Arabidopsis* plants at least in the presence of the repair cycle of PSII [236]. When the protective efficiency of natural NPQ was measured by comparing the rate constant of the damaging reaction of photoinhibition between NPQ-deficient mutants and the wild type, NPQ was found to lower the rate constant of photoinhibition under visible light by 20–30% [52,74]. A somewhat higher protective efficiency was reported from the *psbs* mutant [235].

In order to draw conclusions about the mechanism of photoinhibition, one has to consider the relationship between the

different photoinhibition hypotheses and NPQ. For the acceptor-side mechanism that begins with the formation of stable (double) reduced Q_A, NPQ would slow down both reduction of Q_A and production of $^3P_{680}$ by the acceptor-side-inhibited PSII. The quantum yield of PSII of higher plants (Φ_{PSII}, measured as $\Delta F/F'_M$) is around 0.77 under low light and can drop to 0.1 in high light while the proportion of open PSII centres, measured with the q_P parameter, decreases from ~0.9 to 0.2–0.3 under the same conditions [26,238]. Thus, the quantum yield of open PSII reaction centres and also the quantum yield of formation of stable reduced Q_A would maximally decrease by ~35 to 57% due to light-induced NPQ. Using the q_L parameter [238] instead of q_P would not radically change this conclusion. Because the primary radical pair is in equilibrium with excitations of antenna chlorophylls [239,240], the protective effect of NPQ against formation of $^3P_{680}$ would be directly proportional to the quantum yield of NPQ, which can be more than 70% under high light [238,241]. Due to the combined effect of the two factors, NPQ would lower the quantum yield of acceptor-side photoinhibition under saturating light by 70–80%, compared to the quantum yield of photoinhibition under moderate light.

The protective effect of NPQ against donor-side photoinhibition is more difficult to estimate because the rate of donor-side photoinhibition depends on the steady-state concentration of long-lived P_{680}^+. The protective of NPQ against the formation of the normal, short-lived P_{680}^+ would again be equivalent to the quantum yield of NPQ, or 70–80%. On the other hand, it has been argued that photochemical and non-photochemical quenching can replace each other in protecting PSII against the stochastic type of donor-side photoinhibition [43]. The decrease in photochemical quenching occurring when light intensity is increased would thus explain why the increase in NPQ does not lower the quantum yield of photoinhibition *in vivo* when light intensity increases [30,31,35,36]. However, the quantum yield of the formation of P_{680}^+ is lower in a closed reaction centre than in an open one [239] and therefore photochemical quenching would speed up rather than slow down donor-side photoinhibition.

The low protective efficiency of NPQ is incompatible with the assumption that photoinhibition is fully dependent on light absorbed by PSII antenna. However, the inefficiency of protection by NPQ can be explained either by assuming that photoinhibition occurs by multiple independent mechanisms or by assuming that the chlorophyll antenna has a minor role in the dominating mechanism of photoinhibition.

6. The manganese hypothesis

6.1. Suggested reaction mechanism

The quantum yield of photoinhibition is not dependent on light intensity [2,30,42] and the rate of photoinhibition does not depend on the size of the light-harvesting antenna [45,197,230]. Furthermore, photoinhibition is only slightly affected by quenching of chlorophyll excitations [109–111]. In contrast, the acceptor and donor side hypotheses predict strong dependence of the quantum yield of photoinhibition on light intensity [74] and antenna size and efficient protection by non-photochemical quenching. The $^1O_2$ hypotheses [49,107] and the manganese hypothesis [41,42,51] are attempts to find a reaction mechanism that correctly predicts the reaction kinetic data.

In the manganese mechanism [42] (Fig. 4), excitation of the oxygen-evolving Mn cluster triggers a disturbance of electron transfer from the Mn cluster to P_{680}^+. The inactivation of OEC is associated with the release of an Mn ion from PSII to the lumen. The disturbance of OEC activity is followed by light-induced loss of the residual electron transfer activities of the reaction centre. This second step of the reaction mechanism is mediated by light absorbed by PSII antenna. The second step has little effect on the rate of the overall reaction because PSII that has lost OEC activity loses the residual activity rapidly [40]. Thus, the Mn mechanism predicts an irradiance-independent quantum yield for photoinhibition. The involvement of light absorbed by PSII antenna in the second step explains why the action spectrum of photoinhibition shows low peaks that correspond to light absorption by PSII antenna and why NPQ has a small protective effect. The observation that light absorbed by the chlorophyll antenna has an effect on the rate of photoinhibition implies that the inactivation of OEC is reversible at least to some extent. The evidence for the Mn mechanism will be discussed in more detail below.

6.2. Evidence supporting the participation of manganese in photoinhibition under visible light

6.2.1. Action spectra and physiological effects

The first clue leading to the manganese hypothesis was the observation that the action spectrum of photoinhibition [2,42,47–50] and the absorption spectra of manganese model compounds of OEC [147–149] show similarity over the whole UV–vis spectral range (Fig. 3) [42]. The level of visible-light absorbance of Mn model compounds is in the order or 1% of their maximum UV absorbance. Furthermore, the *in vivo* action spectrum of photoinhibition in the cyanobacterium *Synechocystis* sp. PCC 6803 [50] shows high efficiency of 400–450 nm light and only moderate efficiency of 600–650 nm light although 600–650 nm light is much more efficient in driving electron transport in cyanobacterial PSII [50,242]. These data suggested that the Mn ions of OEC might function as photoreceptors of photoinhibition not only in UV light but also in visible light. The observation that photoinhibition of OEC occurs before the rest of PSII becomes photoinhibited [3,42,51,53] provides further evidence supporting the role of the Mn ions as photoreceptors of photoinhibition.

Fast photoinhibition in mutants in which OEC is affected is circumstantial evidence supporting the importance of the donor side of PSII in photoinhibition. Cyanobacterial mutants lacking the external polypeptides of OEC [243–245], and the barley *chlorina-f2* mutant [246] and the LF-1 mutant of the green alga *Scenedesmus* [207], both with an unstable OEC, are also susceptible to photoinhibition. It should be noted, however, that effects of the mutations on the repair of photoinhibited PSII

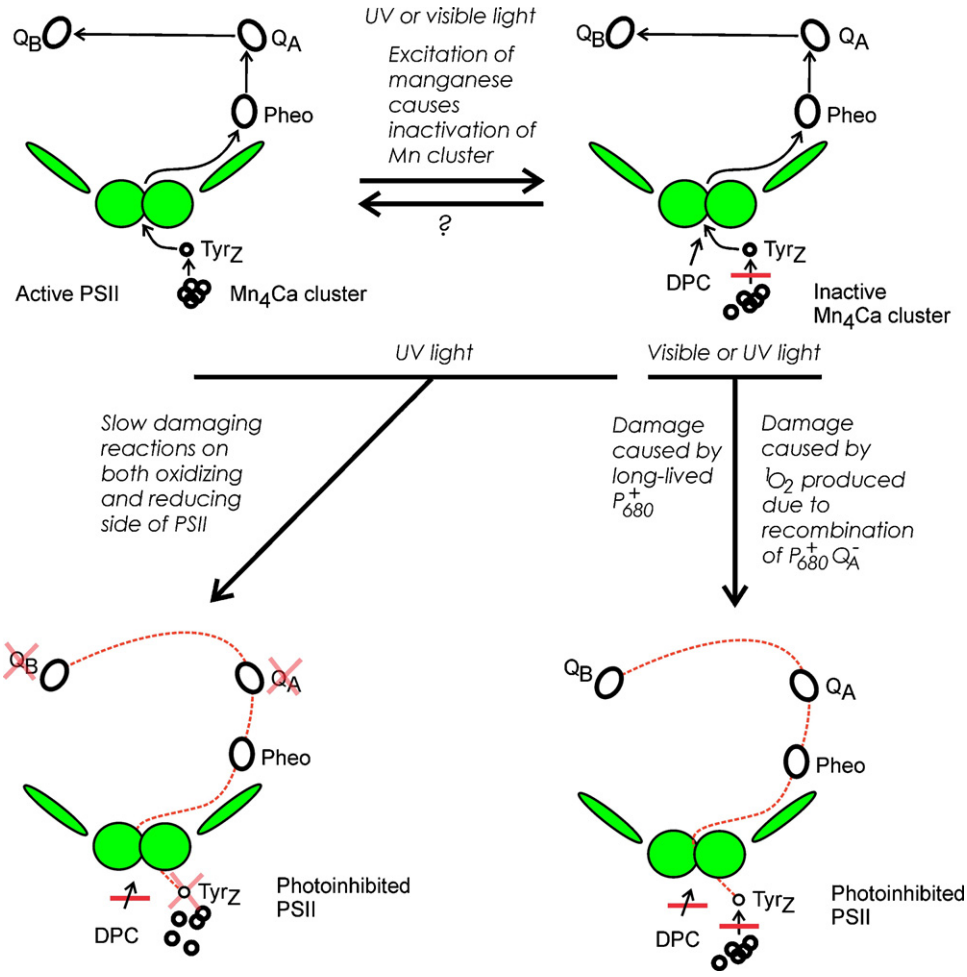

Fig. 4. The manganese mechanism of photoinhibition. Excitation of manganese with UV or visible light converts the Mn cluster to an inactive state which is reversible to some extent. Release of an Mn ion is associated with the inactivation of OEC. When OEC is inactive, the highly oxidizing P_{680}^+ becomes long-lived and may oxidize a wrong component of the reaction centre. Alternatively, recombination of the $P_{680}^+Q_A^-$ charge pair may lead to production of harmful $^1O_2$. If the inactivity of OEC persists, Q_A is converted to the high-potential form which lowers the $^1O_2$ yield. UV light causes additional, slower reactions affecting first TyrZ and the quinone acceptors. The green ellipses depict the four chlorophylls that contribute to P_{680}. Electron transfer routes are shown with arrows between PSII components; the red dashed line indicates non-functional electron transport. UV light causes slower damage to several components of the reaction centre. The ability of DPC to restore PSII electron transport at different phases is shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

were not excluded in the above studies [207,243–246]. As discussed above, chemical treatments like NH_2OH treatment or Tris washing that remove an active Mn cluster, induce rapid photoinhibition (Section 4.2.1). No loss-of-function donor-side mutants are known that protect against photoinhibition.

The release of Mn ion(s) from PSII to the lumen accompanies photoinhibition both in UV light [42,48,57,146] and in visible light [42,62]; mild photoinhibition treatment of PSII membrane fragments did not lead to statistically significant release of manganese [90]. The release of Mn under both visible and UV light suggests that the inactivation of the Mn cluster occurs with a similar mechanism under visible and UV light. However, release of Mn ions to the lumen can obviously result both from light-induced inactivation of OEC and from degradation of the D1 protein, and it is technically difficult to unambiguously distinguish a stoichiometry of one Mn per one inhibited reaction centre [42,48] from a stoichiometry of four Mn ions per one D1 protein degraded [62,146].

6.2.2. *Photosensitivity of manganese enzymes*

To get insight into the eventual photosensitivity of natural model compounds of OEC, we recently compared the effect of UV and visible light on Mn superoxide dismutase and Mn catalase with the effect of light on other enzymes catalyzing the same reactions [158]. In order to limit non-specific effects due to production of reactive oxygen species under UV light, the experiments were done in anaerobic conditions. These experiments revealed that the Mn enzymes were susceptible to photoinhibition at wavelengths absorbed by the Mn cofactors. Photosensitivity does not seem to be a unique property of Mn enzymes, but all metalloenzymes tested were found to be photosensitive at wavelengths absorbed by the metal cofactor complex. Of particular interest is the observation that Mn catalase [150] is sensitive to visible and UV light. This enzyme contains an oxygen-bridged dimanganese active centre [247] and is therefore the closest model of the oxygen-evolving Mn cluster among Mn enzymes. The photoinhibition of Mn catalase

in visible light is apparently caused by light absorbed by the $Mn(III)_2$ centre.

6.3. Chlorophyll driven secondary reactions—P_{680}^+ driven oxidation and singlet oxygen production due to $P_{680}^+Q_A^-$ recombination

Photoinhibition begins with the inhibition of OEC activity and continues with inhibition of other parts of PSII electron transfer chain. The action spectrum of photoinhibition of hydroxylamine-washed thylakoids [42,51] shows that the antenna of PSII is the photoreceptor of the secondary damage. The sequence of events is nicely demonstrated by illuminating thylakoids first with green or UV light and then with red light. These experiments show that inhibition of oxygen evolution with 500 or 310 nm light makes electron transport from DPC to DCPIP sensitive to further inhibition by 680 nm light [51] (Fig. 5). The dependence of the inhibition of oxygen evolution on Mn absorption and the dependence of the secondary reactions on chlorophyll absorption explain why UV and visible light are additive with regard to photoinhibition of oxygen evolution [248]. Synergism between UV and visible light can be seen when the inhibition of electron transfer from DPC to DCPIP [51] (Fig. 5) or D1 protein degradation [127] are studied.

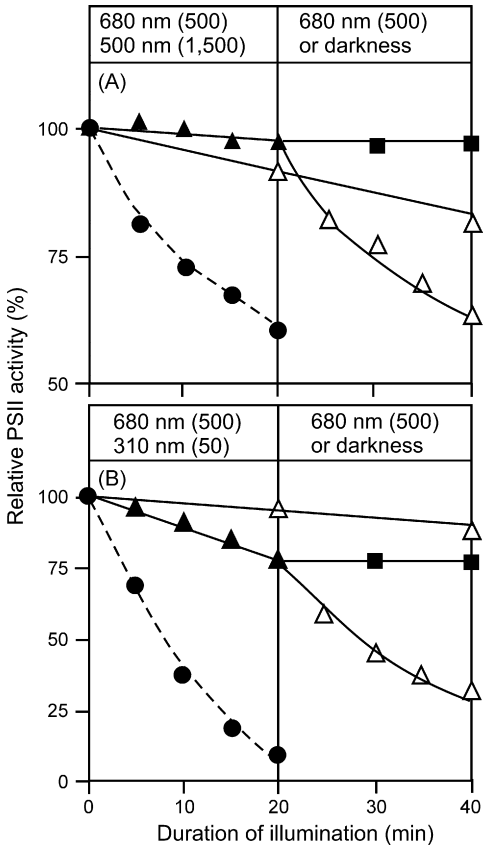

Fig. 5. Demonstration that OEC is damaged in 500 or 310 nm light before electron transport from DCP to DCPIP. Thylakoids of *Thermosynechococcus* were illuminated with 680 nm light (open triangles) (A, B), with 500 nm light (A) (circles and solid triangles) or 310 nm light (B) (circles and solid triangles). After 20 min, samples illuminated at 500 nm (A) or 310 nm (B) were transferred to 680 nm light (open triangles) or darkness (squares). Circles show electron transport activity from H_2O to DCPIP, all other symbols show electron transport activity from DCP to DCPIP. Reprinted with permission from [51] Copyright (2005) American Chemical Society.

The initial versions of the Mn hypothesis [42,51] suggested that the chlorophyll-dependent step in photoinhibition is oxidation of PSII reaction centre components by P_{680}^+. This may be an oversimplification, as several pieces of evidence suggest that the rate of photoinhibition is affected by the probability of production of $^3P_{680}$ by recombination reactions involving Q_A^- (see Section 5.2.2). $^3P_{680}$ can be produced as an intermediate of two types of such recombination reactions: $S_{2/3}Q_A^- \rightarrow S_{1/2}Q_A$ and $P_{680}^+Q_A^- \rightarrow P_{680}Q_A$; it is not known if the reaction $TyrZ^+Q_A^- \rightarrow TyrZQ_A$ produces $^3P_{680}$. The time constant of the $S_{2/3}Q_A^-$ recombination is 1–2 s [249] or even 7 s if measured from thermoluminescence [76]. These time constants are so long that the $S_{2/3}Q_A^-$ recombination saturates at extremely low light and cannot therefore account for photoinhibition under moderate or high light intensities. However, the dominating pathway of $P_{680}^+Q_A^-$ recombination that competes for the reduction of P_{680}^+ during the 'misses' of the S-state cycle has a time constant of 100–200 μs [99,154,155] and can lead to formation of $^3P_{680}$ [99]. I hypothesize that light absorption by the Mn cluster makes the OEC transiently equivalent to an OEC that is about to 'miss' the next turn of the Kok cycle. Such a reaction centre is susceptible to both damage by P_{680}^+ and to damage caused by $^1O_2$ produced due to $P_{680}^+Q_A^-$ recombination.

The involvement of $P_{680}^+Q_A^-$ recombination in photoinhibition is supported by the observation that low pH increases the miss probability [97] and enhances photoinhibition in thylakoids and PSII membranes [71]. However, the irradiance-independent quantum yield of photoinhibition [2,30,31,35,36–38], the low protective efficiency of non-photochemical quenching [37,52,74,109–111], and the preferential inhibition of OEC in photoinhibition [3,42,51,53] indicate that $P_{680}^+Q_A^-$ recombination cannot be alone responsible for photoinhibition but this reaction causes secondary damage after the light-induced disturbance of OEC activity.

Rapid recombination of the $P_{680}^+Q_A^-$ pair occurs only if submicrosecond reduction of P_{680}^+ by TyrZ does not occur, which is most likely when OEC does not function normally [99]. Inactive OEC seems to exclude production of $^1O_2$ because whenever OEC is inactive, the redox potential of the Q_A/Q_A^- pair shifts to a higher value [250–252], and recombination between P_{680}^+ and the high-potential form of Q_A^- no longer favours the thermally activated route that may lead to formation of $^3P_{680}$ via the $P_{680}^+Pheo^-$ pair. However, the shift in the potential of the Q_A/Q_A^- pair is probably not caused by loss of OEC activity *per se* but rather by a protein conformation change that follows the loss of the activity of the Mn complex. Inactivation of OEC would therefore first promote production of $^1O_2$ due to $P_{680}^+Q_A^-$ recombination, but persisting inactivity of OEC induces a conformational change that protects against $^1O_2$ formation. Radical forms of ROS, not $^1O_2$, are therefore mostly found to be produced by PSII both under UV light and under illumination of OEC-less preparates with visible light [70–73,156,157].

Another possible explanation for the apparent importance of the recombination reactions in photoinhibition is to assume that the recombination of the primary radical pair has always a small probability of producing $^3P_{680}$. Such a side reaction of the primary charge separation would constitute a minor, PSII-antenna-dependent photoinhibition pathway and compete with an OEC-dependent mechanism. In this case, the protective efficiency of non-photochemical quenching could be used to calculate the contribution of the PSII-antenna-dependent pathway.

6.4. Is manganese only important in green, blue and UV light?

The preferential inhibition of OEC can be seen if the visible light intensity in the photoinhibition experiment is high [42,53] or if UV, blue or green light is used [42,51] but not in red light [51]. Current experimental data does not allow judging whether red light induces photoinhibition that is entirely chlorophyll-dependent, or whether preferential inhibition of OEC is not seen in red light because the rate of the Mn-dependent step decreases with increasing wavelength while the rate of the chlorophyll-dependent step increases. The shift of the action spectrum of photoinhibition in red light compared to the absorption spectrum of PSII antenna [37] might suggest that the red peak in the action spectrum is superposition of chlorophyll absorption and absorption of another photoreceptor like manganese. The protective effect of NPQ is not stronger in red light than in blue light [52], suggesting that one and the same mechanism dominates photoinhibition in red and blue light.

7. Concluding remarks

The mechanisms of photoinhibition have largely been studied by looking for reactions that would cause inhibition of PSII only under specific conditions like very strong light, very low light, anaerobicity or UVB light, and several specific mechanisms have been found. The phenomenological features of photoinhibition, particularly the fact that the quantum yield of photoinhibition is independent of light intensity, strongly suggest that a single dominating mechanism exists. The acceptor-side hypothesis was long thought to describe such a dominant mechanism, but the linear light response of photoinhibition, the low sensitivity of photoinhibition to PSII excitation pressure and NPQ, and the effect of the redox potential of the Q_A/Q_A^- pair on the rate of photoinhibition cannot be explained with the acceptor-side mechanism. The manganese hypothesis describes a mechanism that can fill these phenomenological requirements but several details of the mechanism remain to be elucidated.

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