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Mechanism of Cd^{2+} toxicity: Cd^{2+} inhibits photoactivation of Photosystem II by competitive binding to the essential Ca^{2+} site

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Abstract

Cadmium (Cd^{2+}) is a well-known highly toxic element. The molecular mechanisms of the Cd^{2+} toxicity are not well understood. In photosynthetic organisms, toxic Cd^{2+} concentrations are often in the low-μM range. It has been proposed that low-μM Cd^{2+} concentrations affect photosynthesis at the level of Photosystem II by inhibiting oxygen evolution. However, in vitro studies on isolated, functional Photosystem II showed that much higher Cd^{2+} concentrations (mM range) were needed for inhibition. Here we show that Cd^{2+} in the low-μM range inhibited photoactivation (i.e., assembly of the water splitting complex) in *Chlamydomonas reinhardtii* and in isolated Photosystem II. Photoactivation is the last step in the assembly of Photosystem II before it becomes functional. The exact Cd^{2+} concentration necessary for inhibition depended on the concentration of calcium. It is proposed that Cd^{2+} binds competitively to the essential Ca^{2+} site in Photosystem II during photoactivation. The low Cd^{2+} concentration needed to inhibit photoactivation suggests that this event is also involved in the Cd^{2+}-induced inhibition of photosynthesis in vivo. This mechanism is likely to be important for Cd^{2+} toxicity towards photosynthetic organisms in general, at least in unicellular like *C. reinhardtii* where Cd^{2+} has easy access to the photosynthetic apparatus.
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Keywords: Photosynthesis; Photosystem II; Photoactivation; Cadmium toxicity

1. Introduction

Cd^{2+} is well known as a highly toxic environmental pollutant that is incorporated and accumulated in large amounts by all organisms so far tested. Although Cd^{2+} toxicity has been studied intensively, the molecular mechanism of this is not well established.

In photosynthetic organisms, Cd^{2+} is readily taken up and affects several metabolic activities in different cell compartments. These effects include inhibition of photosynthesis, binding to chlorophylls, growth inhibition, chlorosis (inhibition of chlorophyll synthesis), decrease in water and nutrient uptake, and root browning (for review, see Refs. [1–5]). Pietrini et al. [6] showed that the Cd^{2+} concentration in the chloroplast of *Phragmites australis* was 0.83 nmol Cd^{2+}/mg chlorophyll when the plants were treated with 50 μM Cd^{2+}. The Cd^{2+} concentrations at which 50% of the inhibition is exerted (called IC_{50} value) depended on the organism as well as the physiological conditions, but was found to be mostly in the low μM (typically 5–30 μM) range (see e.g., Refs. [6–10]). One has to distinguish between short-term and long-term effects of Cd^{2+} exposure, because organisms can adapt to higher Cd^{2+} concentration by employing resistance mechanisms, such as inducing the expression of Cd^{2+}-sequestering compounds (e.g., phytochelatins) or exporters capable of transporting

Abbreviations: Chl, chlorophyll; Fv, variable fluorescence; Fm, maximal fluorescence; MES, 2-(*N*-morpholino)ethansulfoic acid; PSII, Photosystem II

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Cd^{2+} out of the cell (reviewed in Ref. [3]). Also, some mechanism of heavy metal toxicity are slow but nevertheless occur in the low micromolar or even submicromolar range (e.g., Ref. [11]).

Studies in plants, algae and cyanobacteria have shown that a sublethal concentration of Cd^{2+} already inhibits photosynthesis, for which IC_{50} values with a low-μM Cd^{2+} concentration have been reported. In particular, measurements of the inhibition of oxygen evolution by Cd^{2+} resulted in IC_{50} value of 5–10 μM in *C. reinhardtii* [12–14].

Studies in isolated chloroplasts confirmed that photosynthesis was affected by Cd^{2+}. However, the revealed IC_{50} values were significantly higher than in the intact organism, i.e., 1.5–5 mM in chloroplasts isolated from spinach [15] and 0.2 mM in chloroplasts from *Zea mays* [16]. The latter work also indicated that the inhibition of photosynthesis by Cd^{2+} is performed at the donor side of Photosystem II (PSII) (see also Refs. [2,17]), i.e., around the catalytic center of photosynthetic oxygen evolution. More evidence that the inhibitory effect of Cd^{2+} is exerted at the donor site of PSII came from studies of a Cd^{2+}-tolerant mutant of *C. reinhardii* [18]. It was shown that in this Cd^{2+}-tolerant mutant, the donor side of PSII was impaired, indicating first, that the donor side is indeed a site of action for Cd^{2+} inhibition of photosynthesis and second, that this site is one of the first affected by Cd^{2+} in *C. reinhardii*, since this mutant tolerated much higher Cd^{2+} concentrations than the wild type.

Cd^{2+} effects were also studied on isolated PSII membranes from spinach [19–21]. Vrettos et al. [20] showed clearly that Cd^{2+} is a competitive inhibitor of the essential Ca^{2+} site in PSII (see below) and a K_D value of 0.144 mM for the binding of the Cd^{2+} to the Ca^{2+} site has been calculated. The catalytic center of the water oxidation of PSII contains a 4-manganese cluster, a Ca^{2+} ion and 1–2 Cl^- ions as essential cofactors [22–25]. During the assembly of PSII to its functional state, the catalytic center is the last unit introduced. This last step is called photoactivation, because apart from the cofactors Mn^{2+}, Ca^{2+}, and Cl^-, light is also needed. Photoactivation can also be performed in vitro by using isolated, Mn-depleted PSII, to which the cofactors are added. After exposure to light, Photosystem II is photoactivated and can therefore function, i.e., evolves oxygen (see Ref. [26]).

Some organisms, such as green algae, are able to synthesize Chl in the dark and thus assemble stable PSII in the dark lacking the catalytic center for oxygen evolution. Upon exposure to light, PSII is photoactivated. In all PSII-containing organisms, photoactivation is a very frequent process, due to the high turnover of the protein D1, where the catalytic center of PSII is located [27,28].

The present work provides evidence that Cd^{2+} is a competitive inhibitor of the Ca^{2+} site in the catalytic center in PSII during photoactivation. Moreover, the IC_{50} value of Cd^{2+} inhibition is significantly lower during assembly of the catalytic center (photoactivation) than in the assembled (functional) state. This could have an important implications for the mechanism of Cd^{2+} toxicity towards photosynthetic organisms.

2. Materials and methods

2.1. Preparation of PSII particles

PSII-enriched membrane fragments from spinach were prepared according to Ref. [29] with modification as described in Ref. [30]. The activity of the samples was 400–500 μmol O_2 (mg of Chl)$^{-1}$ h^{-1}. They were stored at −80 °C until use.

2.2. NH_2OH treatment

Mn depletion was carried out by incubating PSII particles at a concentration of 0.5 mg Chl/ml in a buffer containing 2 mM NH_2OH, 300 mM sucrose, 15 mM NaCl, and 50 mM MES (pH 6.5) for 1 h in the dark on ice, followed by five washes in the same buffer without NH_2OH. The remaining oxygen evolving activity of the samples was less than 5%.

2.3. In vitro photoactivation of PSII particles

Photoactivation was conducted in a buffer containing 300 mM sucrose, 15 mM NaCl, and 50 mM MES (pH 6.5) using a final Chl concentration of 50 μg ml^{-1}. Samples were incubated for 25 min in glass tubes with a diameter of 0.7 cm at room temperature and illuminated with white light at an intensity of 30 μmol quanta m^{-2} s^{-1}, in the presence of 10 μM 2,6-dichlorphenolindophenol as electron acceptor and 100 μM $MnCl_2$ and 20 mM $CaCl_2$ as typical values unless otherwise stated. Typical O_2-evolution of the photoactivated PSII membranes were 80–130 μmol (mg chlorophyll)$^{-1}$ h^{-1}. The accuracy of the measurement was ±10 μmol O_2 (mg chlorophyll)$^{-1}$ h^{-1}, which is indicated by the error bars (Fig. 2). The relative yield of photoactivation in the presence of different concentrations of $CdCl_2$ was calculated by taking the yield of photoactivation in the absence of $MnCl_2$ and $CaCl_2$ as 0 and the yield of photoactivation in the absence of $CdCl_2$ (but presence of $MnCl_2$ and $CaCl_2$) as 1.

2.4. O_2 evolution measurements

PSII activity was measured at 20 °C with a Clark-type oxygen electrode at pH 6.5 using 0.2 mM *p*-phenylbenzoquinone as electron acceptor and saturating illumination with white light (I=5000 μmol quanta m^{-2} s^{-1}).

2.5. Photoactivation of Chlamydomonas reinhardtii

C. reinhardtii cc124 was grown in the dark on Tris acetate phosphate (TAP) medium for 5 days (Chl content of the cultures: 1.5 μg Chl/ml). This culture was divided into eight aliquots. $CdCl_2$ was added in the dark to a final concentration

in the medium of 0, 1, 10 and 100 μM, respectively. After 1-h incubation in the dark, the *C reinhardtii* were directly measured or exposed to low light intensity (8 μmol quanta m^{-2} s^{-1}) and the time course of the increase of variable fluorescence (Fv/Fm) was measured (for review see Ref. [31]). Chlorophyll fluorescence in vivo was measured with a pulse-amplitude modulation fluorimeter (PAM 101, Walz, Effeltrich, Germany) as described in Ref. [32]. The intensity of the measuring light was low enough to prevent reduction of the plastoquinone pool. Saturating light pulses were given to detect the maximum fluorescence level.

2.6. Photoinhibition of C. reinhardtii

C. reinhardtii cc124 was grown in the light on minimal medium (HSM) medium for 5 days (final Chl content of the culture: 1.5 μg Chl/ml). This culture was divided into eight aliquots. To four of these, $CaCl_2$ was added to a final concentration of 10 mM. Subsequently, $CdCl_2$ was added to three of the Ca^{2+}-enriched and to three of the non-enriched cultures to a final concentration in the medium of 1, 10 and 100 μM $CdCl_2$. After 1-h incubation, each culture was split in two aliquots. One aliquot of each was measured directly whereas the remaining cultures were exposed to high light intensity (1000 μmol quanta m^{-2} s^{-1}) for 1.5 h. Then, the oxygen evolution was measured and they were transferred to low light intensity (8 μmol quanta m^{-2} s^{-1}). After 2 and 6 h, the recovery rate from photoinhibition was measured. Prior to each determination of oxygen evolution, 10 mM $NaHCO_3$ was added and the oxygen consumption was measured for 2.5 min before switching on the actinic light (I=2000 μmol quanta m^{-2} s^{-1}).

3. Results

The time course of the inhibition of oxygen evolution by Cd^{2+} in functional PSII membranes revealed that the inhibition was completed after ~4 h, in agreement with previous findings [20] (data not shown). Fig. 1A shows the concentration dependence of Cd^{2+} inhibition of oxygen evolution after 5-h incubation measured in the absence (filled squares) and presence of 10 mM Ca^{2+} (open circles). The Cd^{2+} concentration needed to inhibit 50% of the oxygen evolving activity of functional PSII membranes (IC_{50} value) was ~8 and ~2.5 mM in the presence of 10 and 0 mM Ca^{2+}, respectively. This is in agreement with values in the low-mM range reported in the literature [15,20] and the fact that increasing the Ca^{2+} concentration can reverse the Cd^{2+} inhibition due to their competitive binding to the same site [20].

Fig. 1, B1, shows the effect of the presence of different Cd^{2+} and Ca^{2+} concentrations on the relative yield of photoactivation in vitro. Photoactivation is the last step in the assembly of functional PSII. This was determined by measuring oxygen evolution of functional PSII after photoactivation. At all Ca^{2+} concentrations measured, photoactivation was inhibited by Cd^{2+} in a concentration-dependent manner. For instance, the relative yield of photoactivation in the presence of 10 mM Ca^{2+} is depicted by the open circles. Here, the IC_{50} value was deduced to be ~0.2 mM. This is 1–2 orders of magnitude lower than for the assembled system (IC_{50} value: 8 mM at the same Ca^{2+} concentration) (Fig. 1A). It can be clearly seen that the change of the Ca^{2+} concentration had a dramatic effect in that in the presence of higher Ca^{2+} concentrations, higher Cd^{2+} concentrations were needed to inhibit the photoactivation and vice versa.

Fig. 1, B2, shows that the IC_{50} values of Cd^{2+} depended directly on the Ca^{2+} concentrations (here shown for concentrations between 0.1 to 20 mM). A linear dependence fitted satisfyingly to the data points, indicating that the IC_{50} value of inhibition of photoactivation by Cd^{2+} can be estimated by 1/50 of the present Ca^{2+} concentration for a broad range of Ca^{2+} concentrations.

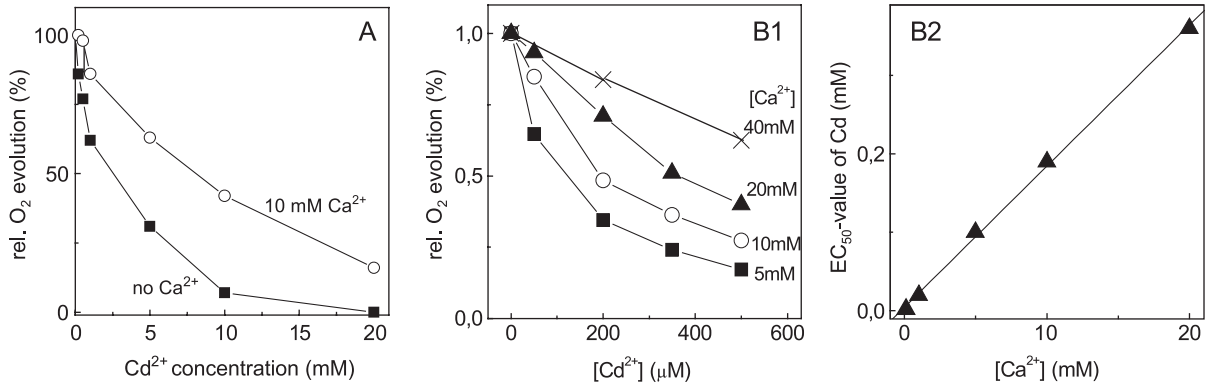

Fig. 1. (A) Cd^{2+} inhibits functional Photosystem II and photoactivation in vitro in a Ca^{2+}-dependent manner. Photoactivation is more sensitive towards Cd^{2+} inhibition. (A) Effect of different Cd^{2+} and Ca^{2+} concentrations on the relative oxygen evolution activity of functional PSII membranes. (B1) Effect of different Cd^{2+} and Ca^{2+} concentration on photoactivation of PSII membranes (expressed as relative oxygen evolution activity). Photoactivation in the presence of 5 mM (filled squares), 10 mM (open circles), 20 mM (filled triangles), and 40 mM (crosses) Ca^{2+}. (B2) Dependence of the IC_{50} values (Cd^{2+} concentration where 50% inhibition of photoactivation occurred) on the Ca^{2+} concentration. The line represents the least-square fit of the data.

In order to analyze the type of inhibition by Cd^{2+} during photoactivation data in Fig. 1, B1, were further treated. Fig. 2A shows these data in a double-reciprocal plot. The lines were obtained by a linear least-square fit for each Cd^{2+} concentration. The fitted lines intersect in an area (see dotted circle Fig. 2A) around the ordinate axis (i.e., at $[Ca^{2+}]^{-1}=0$) indicating that the inhibition of Cd^{2+} is competitive. Whereas an uncompetitive inhibition by Cd^{2+} can be ruled out, the extended area of intersection of the fitted lines do not allow a mixed type inhibition to be ruled out. Mixed type (also called noncompetitive) inhibition shows an intersection of the fitted lines below the ordinate axis (i.e., $[Ca^{2+}]^{-1}<0$). The Dixon plot is shown in Fig. 2B, in which the least-square fitted lines intersect mostly in the second quadrant and the plot of the slopes of these lines against $[Ca^{2+}]^{-1}$ passes through the origin, indicative of competitive inhibition (Fig. 2B, inset) [29].

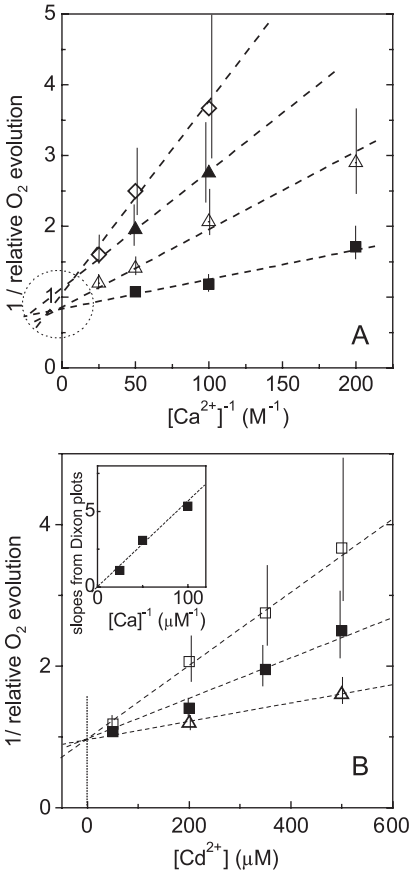

Fig. 2. (A) Evidence that Cd^{2+} is a competitive inhibitor of the essential Ca^{2+} site during photoactivation in vitro. Double-reciprocal plot of the data from Fig. 1, B1: Inverse oxygen evolution activity vs. inverse Ca^{2+} concentration for the inhibition of photoactivation of PSII by 500 μM (open diamonds), 350 μM (filled triangles), 200 μM (open triangles with a dot) and 50 μM (filled squares) Cd^{2+}. (B) Dixon plot of the data from Fig. 1, B1: Inverse oxygen evolution activity vs. Cd^{2+} concentration in the presence of 10 mM (open squares), 20 mM (filled squares) and 40 mM (open triangles) Ca^{2+}. The inset shows the slopes of the lines from the Dixon plot vs. the inverse Ca^{2+} concentration. The dashed lines represent the least-square fits for each concentration of Cd^{2+} and Ca^{2+} for panel A or B, respectively.

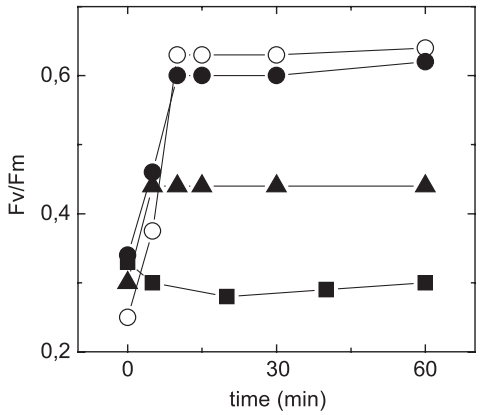

Fig. 3. Cd^{2+} inhibits photoactivation in *C. reinhardtii* in vivo. Plot of variable to maximum fluorescence (Fv/Fm) versus time of light incubation. Fv/Fm is a measure of Photosystem II activity. *C. reinhardtii* were grown in the dark and then incubated with $[Cd^{2+}]$ of 0 (open circles), 1 (filled circles), 10 (triangles), and 100 μM (squares). After 1-h incubation in the dark (time point 0), the *C. reinhardtii* were exposed to white light (8 μmol quanta m^{-2} s^{-1}) and Fv/Fm (reflecting the yield of photoactivation) was followed over 60 min.

In contrast, when the Mn^{2+} concentration was changed (between 10 μM and 1 mM) during photoactivation (Ca^{2+} concentration kept constant), the Cd^{2+} concentration-dependent inhibition (i.e., the IC_{50} values) was not significantly affected (data not shown). This indicates that the inhibition of photoactivation by Cd^{2+} is predominantly due to competition at the Ca^{2+} site and not at the Mn^{2+} sites in PSII.

In order to test these findings in vivo, measurements with whole cells of *C. reinhardti* cc124 were undertaken. *C. reinhardtii* is able (in contrast to most other photosynthetic organisms) to synthesize chlorophylls in the dark and thus assemble PSII up to the point prior to photoactivation (when grown in the presence of acetate as an alternative energy source). Upon exposure to light, PSII in *C. reinhardtii* performs photoactivation, which can be monitored by measuring the quotient of the variable and maximal fluorescence level (for review, see Ref. [31]). Such measurements are shown in Fig. 3 in the presence of different Cd^{2+} concentrations. In the absence of Cd^{2+} (open circles), the photoactivation was completed after a 10-min exposure to light and reached a typical value of 0.6 for Fv/Fm. In contrast, in the presence of 100 μM Cd^{2+}, no photoactivation took place (Fv/Fm stayed constant at around 0.3). In the presence of 10 μM Cd^{2+}, about 40% of the maximal photoactivation was reached, pointing to a IC_{50} value in the low-μM range of Cd^{2+}.

To further investigate the inhibition of photoactivation by Cd^{2+} and its competition with Ca^{2+} in vivo, cells of *C. reinhardtii* cc124 were grown photoautotrophically for 5 days and then subjected to photoinhibitory illumination in the presence of different Cd^{2+} concentrations and in the presence or absence of 10 mM $CaCl_2$ (Fig. 4). Illumination with high light intensity (I=1000 μmol quanta $m^{-2}s^{-1}$)

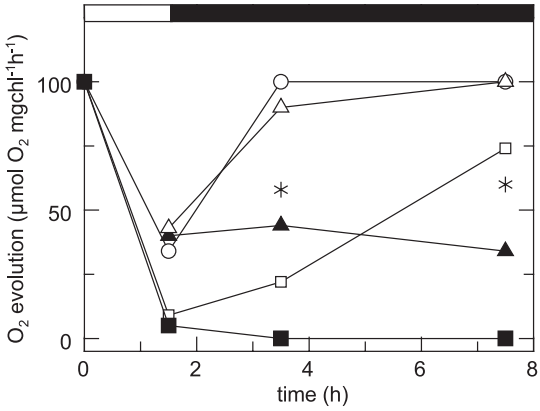

Fig. 4. Ca^{2+} modulates the inhibitory effect of Cd^{2+} on the recovery of PSII after photoinhibition. Plot of oxygen evolution of *C. reinhardtii* cells prior to photoinhibitory illumination, after photoinhibition and during recovery. *C. reinhardtii* were grown in the light (25 µmol quanta m^{-2} s^{-1}) and incubated with [Cd^{2+}] of 0 µM (open circles), [Cd^{2+}] of 10 µM (filled triangles), [Cd^{2+}] of 100 µM (filled squares), 10 mM $CaCl_2$ and 10 µM Cd^{2+} (open triangles), 10 mM $CaCl_2$ and 100 µM Cd^{2+} (open squares). After 1-h incubation (time point 0), the cells were exposed to white light (I=1000 µmol quanta m^{-2} s^{-1}) for 1.5 h (photoinhibition, white bar) and then transferred to weak light (8 µmol quanta m^{-2} s^{-1}) during the recovery phase (black bar). Chloramphenicol (20 µM) was added after the photoinhibitory illumination to samples containing no Cd^{2+} to show the level of recovery without D1 synthesis (asterisk). The oxygen evolution activity prior to photoinhibition was 100–180 µmol O_2 mg chl^{-1} h^{-1} depending on the culture.

inhibited oxygen evolution activity to 40% in the absence of Cd^{2+} and in the presence of 10 µM $CdCl_2$, while in the presence of 100 µM $CdCl_2$, the activity was inhibited to 10% residual activity. In the absence of Cd^{2+}, incubation of the cells in weak light after photoinhibition led to a recovery of oxygen evolving activity, which involved de novo synthesis of the D1 protein, as could be shown by a clear inhibition of recovery in the presence of chloramphenicol (a known inhibitor of D1 synthesis). In the presence of 10 µM Cd^{2+}, recovery did not take place. However, when the medium contained 10 mM Ca^{2+}, efficient recovery occurred. Even in the presence of 100 µM $CdCl_2$, which blocked the recovery completely, restoration of oxygen evolution took place in the presence of 10 mM Ca^{2+} although more slowly than in the presence of lower concentrations of Cd^{2+}. For recovery of photoinhibited PSII, not only is de novo synthesis and reassembly of PSII needed but also, as the last step, photoactivation. The results shown here are most easily interpreted as the inhibitory effect of Cd^{2+} on photoactivation, which can be compensated by Ca^{2+}. When the cells were incubated for 2.5 h with up to 1 mM $CdCl_2$ under low light intensities (25 µmol quanta m^{-2} s^{-1}), no inhibitory effect of Cd^{2+} on oxygen evolution was seen (data not shown). Under these low light conditions, D1 turnover is very slow and hence photoactivation is effectively absent during this 2.5 h. This is in line with the hypothesis that low-µM Cd concentrations inhibit only photoactivation and not assembled PSII.

4. Discussion

This work shows that Cd^{2+} inhibits photoactivation of PSII in vitro in the low-µM range in a Ca^{2+}-sensitive manner. In contrast, Cd^{2+} inhibition of functional PSII in vitro occurred only at ~2 orders of magnitude higher Cd^{2+} concentrations (i.e., low mM range). This seems to be a thermodynamic (and not kinetic) effect, since Cd^{2+} inhibition was assessed at equilibrium (i.e., after 5-h incubation, see Results and Ref. [20]). This clearly underlines the importance of the photoactivation process for the Cd^{2+} inhibition of PSII and suggests that Cd^{2+} inhibition of functional PSII is of little relevance in vivo.

This conclusion is supported by the result that Cd^{2+} inhibition of photoactivation had very similar IC_{50} values in vivo and in vitro (i.e., low-µM range), indicating that the photoactivation experiments in vitro were relevant to what happens in vivo. The comparison is limited by the restricted knowledge about the Ca^{2+} concentration in the lumen and the penetration of Cd^{2+} in the cell and its different compartments, as well as by the possibility that there are more inhibitory processes with similar IC_{50}. However, the lumenal Ca^{2+} concentration is estimated to be in the µM range[1], which would lead to a reasonable IC_{50} value for Cd^{2+} (high nM to low µM) for photoactivation in vivo (based on the result that IC_{50} value for Cd^{2+} is 1/50 of the Ca^{2+} concentration (see Results and Fig. 1, B2).

Published studies have reported IC_{50} values of 5–10 µM Cd^{2+} for oxygen evolution in *C. reinhardii* [12–14] and in the low-µM range in plants, algae and cyanobacteria [7–10]. Although these measurements did not distinguish between photoactivation and functional PSII, it is likely that Cd^{2+} binding during photoactivation played a more important role than Cd^{2+} binding to functional PSII in PSII inhibition. This is also reasonable, since photoactivation is a very frequent process in nature due to the fast turn over of the central protein D1 in PSII [27,28].

Taken together, these results suggest that Cd^{2+} binding to the essential Ca^{2+} site during photoactivation is likely to be an important mode of action in vivo for inhibiting photosynthesis. This mechanism is a serious candidate for the involvement of Cd^{2+} toxicity in photosynthetic organisms in general, at least in unicellular ones, where Cd^{2+} has ready access to the chloroplast [33]. This is corroborated by the work of Voigt and Nagel [18], who

[1] The free Ca^{2+} concentration in the cytosol is well established to be 100–300 nM (for review, see Ref. [35]. In contrast, for the free Ca^{2+} concentration in the stroma, only estimations are available: the basal free Ca^{2+} concentration is ~150 nM in the light and can attain 5–10 µM upon transition to the dark [36]. The description of a pH gradient-dependent Ca^{2+}/H^+ antiporter in the thylakoid membrane (Ca^{2+} transported from the stroma to the lumen) [37] suggests a significantly higher free Ca^{2+} concentration in the lumen, i.e., >150 nM. Since it is conceivable that the origin of Ca^{2+} in the stroma upon transition from light to dark originates from the lumen, the free Ca^{2+} concentration in the lumen can be estimated to be in the µM range.

found a Cd^{2+}-tolerant mutant of *C. reinhardii*, whose impaired donor side of PSII conferred the tolerance of much higher Cd^{2+} concentrations in this mutant compared to the wild type.

The present results suggest that Cd^{2+} is inhibiting photoactivation (and thus the formation of functional PSII) in the low-μM range mostly by binding to the essential Ca^{2+} site. This is the same mechanism as reported for the functional PSII [20], although in that case significantly higher Cd^{2+} concentrations were needed (K_D=0.144 mM). This implies that functional PSII has higher selectivity for Ca^{2+} over Cd^{2+} compared to the binding during photoactivation. Indeed, assembled PSII exhibited a higher binding constant for Ca^{2+} (K_D=0.069 mM) compared to other monovalent (Na, K) and divalent ions (Mn, Ni, Cu, Co, Cd, Sr, Ba) [20]. The lower selectivity of Ca^{2+} over Cd^{2+} during photoactivation could be due to a Ca^{2+} site, which is not yet properly formed [i.e., ligand(s) missing or distorted geometry] or which is relatively flexible to accommodate other metals, both quite reasonable suggestions for a protein in an assembly process.

It could be a general feature that the selectivity of the metal-binding site for the native metal is lower during assembly and/or folding of metalloproteins and thus the protein takes up other metals more easily. These non-native metals might lead to a loss of activity (or gain of abberant activity) of the protein, which could have toxic effects. Thus, metalloproteins would be much more vulnerable towards metal toxicity during assembly/folding. A way to avoid this higher vulnerability is ensured by the metal-chaperoning system, which makes sure that native metals are introduced in already formed metal-binding sites (for recent review, see Ref. [34]).

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References

[1] H. Clijsters, V. Van Assche V., Inhibition of photosyntheis by heavy metals, Photosynth. Res. 7 (1985) 31–40.
[2] Z. Krupta, Cadmium against higher plant photosynthesis—a variety of effects and where do they possibly come from? Z. Naturforsch. 54c (1999) 723–729.
[3] D.H. Nies, Microbial heavy-metal resistance, Appl. Microbiol. Biotechnol. 51 (1999) 730–750.
[4] M.N.V. Prasad, K. Strzałka, Impact of heavy metals on photosynthesis, in: M.N.V. Prasad, J. Hagemeyer (Eds.), Heavy Metal Stress in Plants: From Molecules to Ecosystems, Springer, Berlin, 1999, pp. 117–128.
[5] S. Clemens, M.G. Palmgren, U. Krämer, A long way ahead: understanding and engineering plant metal accumulation, Trends Plant Sci. 7 (2002) 309–315.
[6] F. Pietrini, M.A. Ianelli, S. Pasqualin, A. Massacci, Interaction of cadmium with glutathione and photosynthesis in developing leaves and chloroplasts of *Phragmites australis* (Cav) Trin. ex Steudel, Plant Phys. 133 (2003) 829–837.
[7] A.K. Stobart, W.T. Griffiths, I. Armeen-Bukhari, R.P. Sherwood, The effect of Cd^{2+} on the biosynthesis of chloroplasts in the leaves of barley, Physiol. Plant. 63 (1985) 293–298.
[8] N. Ata, P.P. Saradhi, P. Mohanty, Inhibition of the chloroplast photochemical reactions by treatment of wheat seedlings with low concentrations of cadmium: analysis of electron transport activities and changes in fluorescence yield, Plant Cell Physiol. 32 (1991) 943–951.
[9] H. Schafer, H. Hettler, U. Fritsche, G. Pitzen, G. Roderer, A. Wenzel, Biotests using unicellular algae and ciliates for predicting long-term effects of toxicants, Ecotoxicol. Environ. Saf. 27 (1994) 64–81.
[10] O. Okamoto, C.S. Asano, E. Aidar, P. Colepicolo, Effects of cadmium on growth and superoxide dismutase activity of the marine microalga *Tetraselmis gracilis* (Prasinophyceae), J. Phycol. 32 (1996) 74–79.
[11] H. Küpper, F. Küpper, M. Spiller, Environmental relevance of heavy metal substituted chlorophylls using the example of water plants, J. Exp. Bot. 47 (1996) 259–266.
[12] U. Irmer, Wirkung der Schwermetalle Cadmium und Mangan auf einzellige Süsswasseralgen im Durchflussystem, Mitt. Inst. Allg. Bot. Hambg. 19 (1984) 5–21.
[13] K. Nagel, J. Voigt, Impaired photosynthesis in a cadmium-tolerant *Chlamydomonas* mutant strain, Microbiol. Res. 150 (1995) 105–110.
[14] F.G. Rott, Entwicklung eines mikrosensorischen Testsystems für ökotoxikologische und physiologische Untersuchungen unter Verwendung der einzelligen Grünalge *Chlamydomonas reinhardtii*, Dissertation, Biologie, Universität Freiburg, 2000.
[15] M.A. van Duijvendijk Matteoli, G.M. Desmet, On the inhibitory action of cadmium on the donor side of photosystem II in isolated chloroplasts, Biochim. Biophys. Acta 408 (1975) 164–169.
[16] M.B. Bazzaz, Govindjee, Effects of lead chloride on chloroplast reactions, Environ. Lett. 6 (1974) 1–12.
[17] J. Voigt, K. Nagel, D. Wrann, A cadmium tolerant *Chlamydomonas* mutant strain impaired in photosystem II activity, J. Plant Physiol. 153 (1998) 566–573.
[18] J. Voigt, K. Nagel, The donor-side of photosystem II is impaired in a Cd^{2+}-tolerant mutant strain of the unicellular green alga *Chlamydomonas reinhardtii*, J. Plant Physiol. 159 (2002) 941–950.
[19] C.M. Waggoner, C.F. Yocum, Calcium activated oxygen evolution, in: M. Baltscheffsky (Ed.), Current Research in Photosynthesis, vol. I, Kluwer, Netherlands, 1990, pp. 733–736.
[20] J.S. Vrettos, D.A. Stone, G.W. Brudvig, Quantifying the ion selectivity of the Ca^{2+} site in photosystem II: evidence for direct involvement of Ca^{2+} in O_2 formation, Biochemistry 40 (2001) 7937–7945.
[21] K.G.V. Sigfridsson, G. Bernát, F. Mamedoy, S. Styring, Molecular interference of Cd^{2+} with photosystem II, Biochim. Biophys. Acta 1659 (2004) 19–31.
[22] R.J. Debus, Amino acid residues that modulate the properties of tyrosine Y(Z) and the manganese cluster in the water oxidizing complex of photosystem II, Biochim. Biophys. Acta 1503 (2001) 164–186.
[23] A. Zouni, H.T. Witt, J. Kern, P. Fromme, N. Krauss, W. Saenger, P. Orth, Crystal structure of photosystem II from *Synechococcus elongatus* at 3.8 A resolution, Nature 409 (2001) 739–743.

[24] A.W. Rutherford, P. Faller, The heart of photosynthesis in glorious 3D, Trends Biochem. Sci. 26 (2001) 341–344.
[25] K.N. Ferreira, T.M. Iverson, K. Maghlaoui, J. Barber, S. Iwata, Architecture of the photosynthetic oxygen-evolving center, Science 303 (2004) 1831–1838.
[26] G.M. Ananyev, L. Zaltsman, C. Vasko, G.C. Dismukes, The inorganic biochemistry of photosynthetic oxygen evolution/water oxidation, Biochim. Biophys. Acta 1503 (2001) 52–68.
[27] A. Prasil, N. Adir, I. Ohad, Dynamics of photosystem II: mechanism of photoinhibition and recovery process, in: J. Barber (Ed.), The Photosystems: Structure, Function and Molecular Biology, Elsevier, Amsterdam, 1992, pp. 295–348.
[28] B. Andersson, E.M. Aro, Proteolytic activities and proteases of plant chloroplasts, Physiol. Plant. 100 (1997) 780–793.
[29] D.A. Berthold, G.T. Babcock, C.F. Yocum, A highly resolved, oxygen-evolving photosystem II preparation from spinach thylakoid membranes, FEBS Lett. 134 (1981) 231–234.
[30] G.N. Johnson, A. Boussac, A.W. Rutherford, The origin of 40–50 °C thermoluminescence bands in photosystem II, Biochim. Biophys. Acta 1184 (1994) 85–92.
[31] K. Maxwell, G.N. Johnson, Chlorophyll fluorescence—a practical guide, J. Exp. Bot. 51 (2000) 659–668.
[32] U. Schreiber, U. Schliwa, W. Bilger, Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer, Photosynth. Res. 10 (1986) 51–62.
[33] K. Nagel, U. Adelmeier, J. Voigt, Subcellular distribution of cadmium in the unicellular green alga *Chlamydomonas reinhardtii*, J. Plant Physiol. 149 (1996) 86–90.
[34] L.A. Finney, T.V. O'Halloran, Transition metal speciation in the cell: insights from the chemistry of metal ion receptors, Science 300 (2003) 931–936.
[35] D. Sanders, C. Brownlee, J.F. Harper, Communicating with calcium, Plant Cell 11 (1999) 691–706.
[36] C.H. Johnson, M.R. Knight, T. Kondo, P. Masson, J. Sedbrook, A. Haley, A. Trewavas, Circadian oscillations of cytosolic and chloroplastic free calcium in plants, Science 269 (1995) 1863–1865.
[37] W.F. Ettinger, A.M. Clear, K.J. Flanning, M.L. Peck, Identification of a Ca^{2+}/H^{+} antiport in the plant chloroplast thylakoid membrane, Plant Physiol. 119 (1999) 1379–1385.