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Molecular mechanism of action of Pb^{2+} and Zn^{2+} on water oxidizing complex of photosystem II

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Abstract

Pb^{2+} and Zn^{2+} inhibition of photosystem II (PSII) activity was reported to be mediated via displacement of native inorganic cofactors (Cl^-, Ca^{2+} and Mn^{2+}) from the oxygen evolving complex, OEC [Rashid and Popovic (1990) FEBS Lett. 271, 181–184; Rashid et al. (1991) Photosynth. Res. 30, 123–130]. Since the binding sites of these cofactors are protected by a shield of three extrinsic polypeptides (17, 23 and 33 kDa), we investigated whether these metal ions affect the extrinsic polypeptide shield of OEC. By immunoblotting with antibodies recognizing the 23 and 33 kDa polypeptides, we showed that both the metal ions significantly dissociated the 23 kDa (+17 kDa) polypeptide, and partially dissociated the 33 kDa. Ca^{2+}, one of the important inorganic cofactors of oxygen evolution, strongly prevented the dissociating action of Pb^{2+} but did not prevent the action of Zn^{2+}. The probable molecular mechanism of action of Pb^{2+} and Zn^{2+} on PSII OEC is discussed.

Key words: Photosystem II; Oxygen evolving complex; Extrinsic polypeptides; Immunoblotting; Lead action; Zinc action

1. Introduction

Both Pb^{2+} and Zn^{2+} are environmental contaminants. At slightly elevated levels, they severely inhibit green plant photosynthesis [1]. Studies on isolated chloroplasts have shown that the site of action of Pb^{2+} and Zn^{2+} is on the water oxidizing side of PSII [2,3,4]. This side of PSII is located within the closed lumenal surface of thylakoid membranes, and composed of three extrinsic polypeptides of approximate molecular masses 17, 23 and 33 kDa. At least three inorganic cofactors, Cl^-, Ca^{2+} and Mn^{2+}, are reported to be associated with these polypeptides (see reviews by [5,6,7,8]). By using detergent-derived PSII membrane fractions, in which the donor side is exposed to outer environment [9], it was shown that Pb^{2+} competitively inhibited Ca^{2+} and Cl^- binding [10], and Zn^{2+} non-competitively inhibited Ca^{2+} and Mn^{2+} binding [11] at native sites on water oxidase. From these studies, it was concluded that Pb^{2+} or Zn^{2+}-induced inhibition of photosystem II activity was mediated by the loss of specific inorganic cofactors, necessary for water oxidation [10,11].

However, it is well established that the cofactor binding sites in OEC are protected by a shield of extrinsic polypeptides [6,7]. Treatments which extract cofactors may also deplete membrane's extrinsic polypeptides. For example, treatment with high concentration of NaCl removes part of native Ca^{2+} and also removes 17 and 23 kDa polypeptides [12,13]. Likewise, extraction of Mn^{2+} occurs during treatment with alkaline Tris (pH > 9.0) which removes relatively tightly bound 33 kDa polypeptide [14–16].

From the fact that Pb^{2+} competes competitively with Ca^{2+} and Cl^- at binding sites [10], which are protected by the 17 and 23 kDa polypeptides [6,7], we predicted that the action of Pb^{2+} also includes depletion of OEC polypeptides. On the other hand, since Zn^{2+} inhibition of Ca^{2+}-binding is suggested to involve conformational changes in the PSII core complex [11], it seemed possible that Zn^{2+}-binding might occur at a site that did not involve depletion of the extrinsic polypeptides.

In this report, we further investigated the effect of Pb^{2+} and Zn^{2+} on the extrinsic polypeptides of OEC, using high affinity monospecific antibodies to assess depletion of 23 and 33 kDa oxygen evolving polypeptides of PSII. To our knowledge, this is the first report which shows that both Pb^{2+} and Zn^{2+} have strong dissociating action on oxygen evolving extrinsic polypeptides of PSII.

2. Materials and methods

PSII membranes were isolated from spinach (*Spinacea oleracea* L.) following [17]. Concentrated suspensions of the preparations (7.5 mg Chl/ml) were stored at −80°C in a medium containing 0.4 M sucrose, 2 mM NaCl, 25 mM Mes-NaOH (pH 6.0) as described previously [18].

The concentration dependence of Pb^{2+} or Zn^{2+} action on oxygen evolving polypeptides of PSII was studied as follows: the PSII membranes were thawed and washed with a buffer containing 25 mM Mes-NaOH (pH 6.0). They were then suspended in the same buffer with a Chl conc. of 20 $\mu g \cdot ml^{-1}$ and incubated with different concentrations (0, 2, 5 and 10 mM) of either $Pb(NO_3)_2$ or $Zn(NO_3)_2$ for 1.5 h in darkness

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Abbreviations: PSII, photosystem II; OEC, oxygen evolving complex; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis.

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on ice. The PSII membranes were then collected separately by centrifugation at 39,000 × g for 30 min and finally suspended in a buffer containing 0.4 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$, 25 mM Mes-NaOH (pH 6.0). Other conditions are mentioned in figure legends.

The pH dependence of Pb^{2+} or Zn^{2+} action on oxygen evolving polypeptides of PSII was carried out following the same protocol, as described above except that three different pH levels, 5.5, 6.0 and 6.7 of the media were used. For details, see Fig. 2 legend. After the treatments, the membranes were collected and finally suspended as mentioned above.

SDS-PAGE (12% acrylamide gradient gel) followed by immunoblotting was carried out as in [19]. Antibodies to the 33 kDa polypeptide of OEC of spinach were the gift of P. Ann K. Eastman. Antibodies which recognized the 23 kDa polypeptide of the OEC were prepared by immunization of rabbits with a conjugate of carrier protein (keyhole limpet hemocyanin) plus the following synthetic polypeptide: AY-GEAANVFGAPKKNTDFITC (Multiple Peptide Systems, San Diego). The first twenty amino acids were part of the N-terminal amino acid sequence of the western white pine PSII protein *Pin m* I, which bears considerable sequence homology to similar polypeptides from a number of species [20]. Each antiserum reacted monospecifically with immunoblots on nitrocellulose (data not shown). A mixture of the two antisera was prepared to permit localization of both OEC polypeptides on the same lanes.

3. Results and discussion

Fig. 1 shows that incubation of PSII membranes with 2, 5 and 10 mM $Pb(NO_3)_2$, resulted in significant depletion of 23 kDa oxygen evolving extrinsic polypeptide (lanes 2, 3 and 4, respectively), but only partially depleted the 33 kDa polypeptide. Similar results were obtained when $PbCl_2$ was substituted for $Pb(NO_3)_2$, indicating that Pb^{2+} was the active dissociating agent. The pattern of depletion of the above polypeptides by three increasing concentrations of $Pb(NO_3)_2$ was similar, suggesting that Pb^{2+} concentration less than 2 mM was effective. However, when 10 mM $Ca(NO_3)_2$ was present along with 10 mM $Pb(NO_3)_2$, both 23 and 33 kDa polypeptides were retained in the membranes (Fig. 1, lane 5). $CaCl_2$ could not be used because it reacted with $Pb(NO_3)_2$ producing white precipitations. Other tested ions (Mg^{2+}, Mn^{2+}, Cl^-) were ineffective in protecting Pb^{2+}-induced dissociation of polypeptides. The action of Ca^{2+} in counteracting Pb^{2+} effect is consistent with its role in preventing inhibition of PSII activity by Pb^{2+} [10]. A comparable protective action of Ca^{2+} against La^{3+}-induced dissociation of 33 kDa extrinsic polypeptide was also reported earlier [21].

Fig. 1 (lanes 6, 7 and 8) further shows that treatment of PSII membranes with 2, 5 and 10 mM $Zn(NO_3)_2$ resulted in depletion of oxygen evolving polypeptides. The pattern of depletion by $Zn(NO_3)_2$ was more or less similar to the pattern of depletion by $Pb(NO_3)_2$. Similar results were obtained when $ZnCl_2$ was substituted for $Zn(NO_3)_2$, suggesting that the effect was attributed to Zn^{2+}. Ca^{2+} did not play any protective role against Zn^{2+}-induced dissociation of polypeptides (lane 9). This is different from its role against the dissociation of polypeptides by Pb^{2+} (see lane 5). These differential effects of Ca^{2+} against Pb^{2+} and Zn^{2+}-induced dissociation of

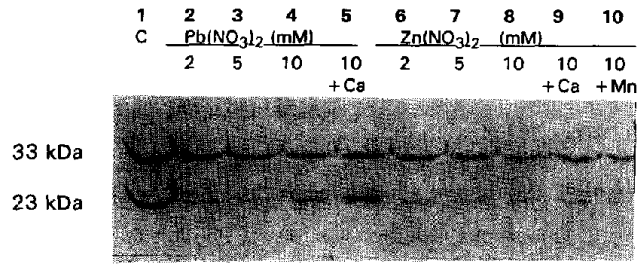

Fig. 1. Immunoblot showing the effects of different concentrations of Pb- and Zn-salts on the oxygen evolving polypeptides of PSII. Concentrations of $Pb(NO_3)_2$: lane 1 = 0 mM; lane 2 = 2 mM; lane 3 = 5 mM; lane 4 = 10 mM; lane 5 = 10 mM + 10 mM $Ca(NO_3)_2$. Concentrations of $Zn(NO_3)_2$: lane 6 = 2 mM; lane 7 = 5 mM; lane 8 = 10 mM; lane 9 = 10 mM + 10 mM $Ca(NO_3)_2$; lane 10 = 10 mM + 10 mM $MnCl_2$.

polypeptides, might be well related to its 'competitive' and 'non-competitive' binding interactions, reported earlier with Pb^{2+} and Zn^{2+}, respectively [10,11].

It has been seen in previous experiments that at elevated pH levels (> 7.0), Zn^{2+} produced insoluble $Zn(OH)_2$ [11]. In order to ascertain that the extrinsic polypeptide dissociating effectiveness of Pb^{2+} and Zn^{2+} was not associated with any secondary effect we studied the action of Pb^{2+} and Zn^{2+} in dissociating the above polypeptides from PSII membranes at three different pH levels (5.5, 6.0 and 6.7) of the media (Fig. 2). The pH of the suspending media, ranging from 5.5 to 6.7, did not appear to influence the polypeptide dissociating effectiveness of Pb^{2+} (lanes 2, 5 and 8, respectively) or Zn^{2+} (3, 6 and 9, respectively), or polypeptide content in the absence of Pb^{2+} or Zn^{2+} (lanes 1, 4 and 7, respectively). It was separately confirmed that no chemical modification of Pb^{2+} or Zn^{2+} salt occurs at our experimental pH range (pH 5.5–6.7).

While the dissociation of the 17 kDa polypeptide was not specifically examined in this study, since the 17 kDa polypeptide cannot bind to OEC unless 23 kDa is already present at its site (see review by [6]), we assume that a concomitant dissociation of 17 and 23 kDa polypeptides is inevitable in the presence of Pb^{2+} or Zn^{2+}. Since Ca^{2+} prevents Pb^{2+}-induced inhibition of PSII activity [10], as well as depletion of 23 kDa (and 17 kDa) extrinsic polypeptide(s) reported here, we suggest that polypeptide removal may be key to the destabilization of PSII OEC by Pb^{2+}. On the other hand, depletion of 23 kDa (and 17 kDa) polypeptide(s) by Zn^{2+} is not prevented in the presence of Ca^{2+}. This is consistent with the previous observations that inhibition of PSII activity by Zn^{2+} was biphasic, and that Ca^{2+} was effective in protecting the activity of the high affinity site (binding constant 0.6 mM) [11]. A lower affinity site (6.6 mM) was much less affected by the presence of Ca^{2+}, and may be involved in the action of Zn^{2+} in depleting the 23 kDa (and 17 kDa) polypeptide(s) reported here.

Thus, the results presented in this report clearly show that both Pb^{2+} and Zn^{2+} have the properties of dissociat-

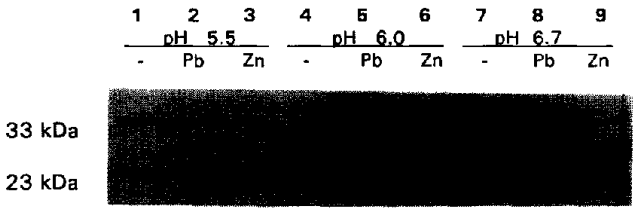

Fig. 2. Immunoblot showing the effects of Pb- and Zn-salts on the oxygen evolving polypeptides of PSII at different pH levels. Lanes 1–3 = pH 5.5; lanes 4–6 = pH 6.0; lanes 7–9 = pH 6.7. 0 mM (lanes 1, 4 and 7); 10 mM $Pb(NO_3)_2$ (lanes 2, 5 and 8); 10 mM $Zn(NO_3)_2$ (lanes 3, 6 and 9).

ing oxygen evolving polypeptides from PSII. Since dissociation of these polypeptides from OEC not only inhibits the activity of water oxidizing enzyme, but also destabilizes the binding of cofactors (Cl^-, Ca^{2+} and Mn^{2+}) in OEC [5,6,7], we suggest this to be a probable molecular mechanism of action of these metal ions on PSII.

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