Protective role of $CaCl_2$ against Pb^{2+} inhibition in Photosystem II

Abdur Rashid and Radovan Popovic

Département de chimie, Université du Québec à Montréal, Case postale 8888, Succ. 'A', Montréal, Québec, H3C 3P8, Canada

Received 3 August 1990)

The Pb^{2+} treatment of Photosystem II (PS II) membrane fragments, either intact or depleted in 17 and 23 kDa extrinsic polypeptides, inhibits PS II activity. When $CaCl_2$ was present in the assay, the Pb^{2+} inhibition was significantly reduced in both types of PS II membranes, suggesting a protective role of $CaCl_2$ against Pb^{2+} inhibition. However, in either case, the degree of PS II inhibition by Pb^{2+} was higher in the protein depleted than in intact PS II. It showed that the loss of endogenous Ca^{2+} induced by polypeptide depletion causes the PS II to be more susceptible to Pb^{2+}. The interaction of Pb^{2+} with $CaCl_2$ in protein-depleted PS II was competitive. Our results suggest that Pb^{2+} competes for binding to the Ca^{2+}- and Cl^- active sites in the water-splitting complex. Since Pb^{2+} inhibition of PS II activity cannot be reversed by $CaCl_2$ but can be reversed by diphenylcarbazide, we conclude that Pb^{2+} induced inhibition of PS II activity was mediated via the water-splitting system.

Photosystem II; Lead inhibition; Calcium chloride; Oxygen evolution; Electron transport; Extrinsic polypeptide; Variable fluorescence

1. INTRODUCTION

The toxic effect of Pb^{2+} on plants has been reported to cause an inhibition of photosynthetic electron transport in chloroplasts. The Photosystem II electron transport was found to be more affected by Pb^{2+} than Photosystem I. The inhibition by Pb^{2+} was indicated to be located at the water-splitting complex (WSC) [1]. It was previously found that Ca^{2+} prevented the similar inhibition by lanthanide ions in PS II. It appeared that lanthanide ions might interfere with the role of Ca^{2+} in WSC [2]. It was shown that the Ca^{2+}- and the Cl^--binding sites in the WSC were protected by a shield of 17 and 23 kDa extrinsic polypeptides [3].

Since the inhibitory effect of Pb^{2+} is associated with the WSC [1], Pb^{2+} may interfere with Ca^{2+}- or Cl^--related functions. In the present study, we made an attempt to elucidate the relationship between Pb^{2+} inhibition phenomenon and the PS II Ca^{2+} and Cl^- dependency functions.

2. MATERIALS AND METHODS

PS II membrane fragments were isolated from spinach according to Berthold et al. [4] and with modifications as described previously [5]. The depletion of 17 and 23 kDa polypeptides from PS II fragments was done as in [6]. The PS II membrane fragments (2 mg Chl/ml), either protein depleted or intact, were stored in 20 mM Mes-NaOH (pH 6.3) buffer.

Oxygen evolution was measured at 22°C with a Clark-type electrode in a medium containing 20 mM Mes-NaOH (pH 6.3) and 0.5 mM DCBQ as PS II electron acceptor and 20 μg Chl/ml.

Photoreduction of DCIP by PS II membrane fragments was done in a medium containing 20 mM Mes-NaOH (pH 6.3), 30 μM DCIP and 6 μg Chl/ml [7].

Chlorophyll fluorescence induction of PS II membrane fragments was monitored following [8] in the same buffer medium (without DCIP) containing 5 μg Chl/ml.

Correspondence address: R. Popovic, Département de Chimie, Université du Québec à Montréal, Case postale 8888, Succursale 'A', Montréal, Québec H3C 3P8, Canada

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; Mes, 2-(*N*-morpholine)ethanesulfonic acid; DPC, diphenylcarbazide; DCBQ, 2,5-dichlorobenzoquinone; WSC, water-splitting complex; Chl, chlorophyll; PS, photosystem

3. RESULTS AND DISCUSSION

In PS II membrane fragments, either intact or depleted in 17 and 23 kDa extrinsic polypeptides, a strong inhibitory effect of Pb^{2+} on oxygen evolution was noticed when $CaCl_2$ was absent. The concentration of Pb^{2+} required to inhibit 50% of oxygen evolution was lower in protein-depleted than in intact PS II membranes. This showed that PS II was more sensitive to Pb^{2+} when extrinsic 17 and 23 kDa polypeptides were depleted from the WSC. In either case, when $CaCl_2$ was absent, 0.2 mM Pb^{2+} was enough to completely inhibit the oxygen evolution. On the other hand, when $CaCl_2$ (15 mM) was added along with Pb^{2+}, the inhibitory action of Pb^{2+} on oxygen evolution was significantly reduced. This demonstrates that the presence of exogenous $CaCl_2$ prevented the PS II inhibition by Pb^{2+}. However, in protein-depleted PS II, the preventive effect of $CaCl_2$ against Pb^{2+} inhibition was smaller than in intact PS II (Fig. 1). The inhibition was confined to Pb^{2+}, since similar effects were found when $Pb(NO_3)_2$ was substituted by $PbCl_2$ (not shown).

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

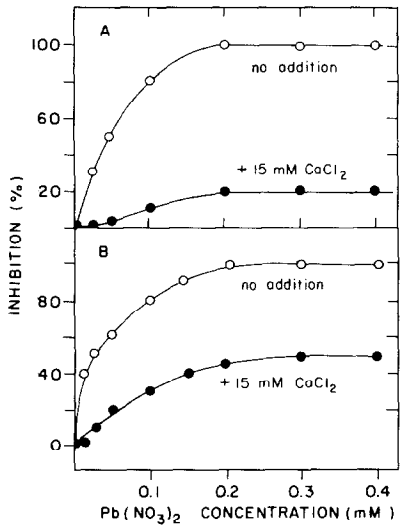

Fig. 1. Inhibition of oxygen evolution ($H_2O \longrightarrow$ DCBQ) by $Pb(NO_3)_2$ in the absence (○) and in the presence (●) of $CaCl_2$ (15 mM). (A) Intact PS II. (B) NaCl-washed PS II. Absolute values for oxygen evolution activity: (A) no addition 366 and +15 mM $CaCl_2$ 402 μmol O_2/mg Chl/h; (B) no addition 107 and +15 mM $CaCl_2$ 350 μmol O_2/mg Chl/h.

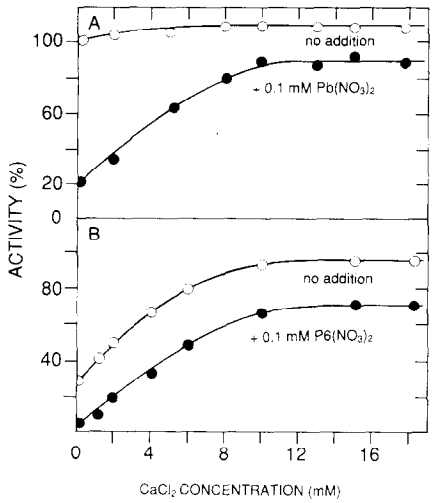

Fig. 2. Dependence of the oxygen evolution activity ($H_2O \longrightarrow$ DCBQ) to $CaCl_2$ concentrations in the absence (○) and in the presence (●) of $Pb(NO_3)_2$ (0.1 mM). (A) Intact PS II; (B) NaCl-washed PS II. For details see legend to Fig. 1.

The effect of $CaCl_2$ concentration on PS II inhibition by Pb^{2+} was examined. The protein-depleted PS II membranes treated with 0.1 mM Pb^{2+} (in absence of $CaCl_2$) retained only 4% oxygen evolving activity. When the concentration of $CaCl_2$ was increased in the presence of 0.1 mM Pb^{2+}, the PS II activity was progressively restored and reached up to 70% at 15 mM $CaCl_2$ (Fig. 2B). However, the PS II membranes depleted in two extrinsic polypeptides, not treated with Pb^{2+}, still retained 29% of the oxygen evolving activity. This activity can be restored up to 96% upon addition of $CaCl_2$ (15 mM). Similar properties of PS II depleted in 17 and 23 kDa polypeptides were also observed earlier [6,9]. On the other hand, the intact PS II membranes treated with 0.1 mM Pb^{2+}, retained 20% of the oxygen evolving activity. When $CaCl_2$ was added along with 0.1 mM Pb^{2+}, up to 90% of the PS II activity was progressively restored. The observed stimulation of oxygen evolving activity (10%) by $CaCl_2$ in intact PS II, not treated with Pb^{2+} was probably a recovery phenomenon, since the measuring medium was devoid of Cl^- (Fig. 2A). These results confirm that $CaCl_2$ has a preventive action against Pb^{2+} inhibition in PS II. This preventive effect of $CaCl_2$ was weaker in protein depleted than in intact PS II. Since we washed the PS II membrane fragments with NaCl (1.5 M) in order to deplete 17 and 23 kDa extrinsic polypeptides, a concomitant partial loss of endogenous Ca^{2+} was induced [10]. Therefore, we suppose that the higher sensitivity of the protein depleted PS II to Pb^{2+} compared to the intact PS II, even in the presence of exogenous $CaCl_2$, is a consequence of the important loss of the endogenous Ca^{2+} induced by polypeptide depletion. It seems that the loss of endogenous Ca^{2+} from the WSC cannot be fully compensated by the added Ca^{2+}. As $CaCl_2$ can prevent Pb^{2+} inhibition in protein-depleted PS II, 17 and 23 kDa polypeptides are probably not directly involved in Pb^{2+} inhibition phenomena. Our results suggest that $CaCl_2$ has an antagonistic effect against Pb^{2+} inhibition in PS II. In protein-depleted PS II, when $CaCl_2$ was progressively added in the presence of 0.1 mM Pb^{2+}, a competitive interaction between Pb^{2+} and $CaCl_2$ was also demonstrated (Fig. 3).

Aside from Ca^{2+} and Cl^-, we have investigated the possible effects of other ions (Na^+, Mn^{2+}, Mg^{2+}, NO_3^-, SO_4^-) in preserving the PS II activity from Pb^{2+} inhibition. We observed that no ions, other than Ca^{2+} and Cl^-, were able to prevent Pb^{2+} inhibition in PS II. Both Ca^{2+} and Cl^- could separately preserve the PS II activity from Pb^{2+} inhibition. However, a combination of the two ions ($CaCl_2$) produced a stronger protection against Pb^{2+} inhibition (Table I).

The variable fluorescence yield of the intact PS II membranes was highly quenched in the presence of 0.1 mM Pb^{2+}. This quenching effect indicates that Pb^{2+} inhibition is associated with the oxygen-evolving complex [11,12]. When $CaCl_2$ (15 mM) and Pb^{2+} (0.1 mM) were combined, the fluorescence yield was significantly preserved, indicating again that $CaCl_2$ has a protective action against Pb^{2+} inhibition on PS II (Fig. 4). As the control sample was devoid of Cl^-, addition of $CaCl_2$ increased the initial rise of variable fluorescence. Since DPC in the presence of Pb^{2+} restored either the yield of variable fluorescence by 85% (Fig. 4) or the photoreduction of DCIP by 80% (result not shown), it appears that the principal inhibitory site of Pb^{2+} is at WSC.

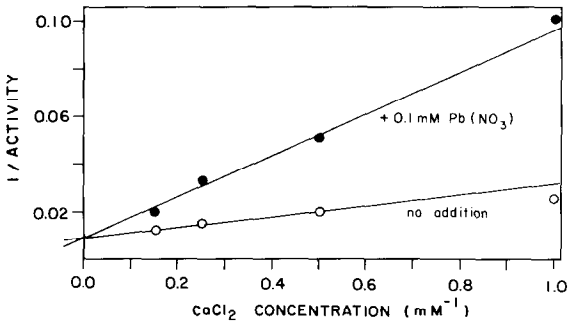

Fig. 3. Double reciprocal plot of the competitive interaction between $Pb(NO_3)_2$ and $CaCl_2$ in NaCl-washed PS II membranes. No addition (○) and presence of $Pb(NO_3)_2$ (0.1 mM) (●).

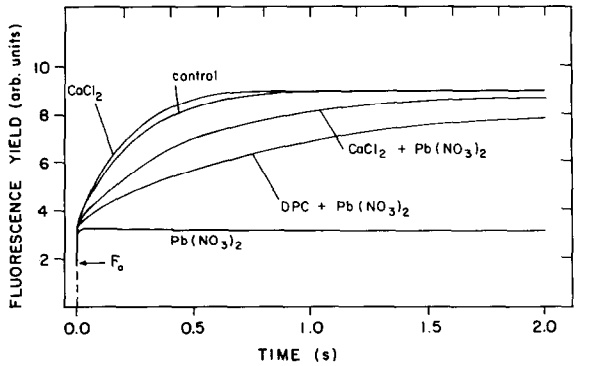

Fig. 4. Fluorescence induction kinetics in intact PS II membranes. The concentration of additions: 0.1 mM $Pb(NO_3)_2$; 15 mM $CaCl_2$ and 0.5 mM DPC.

In the presence of 15 mM $CaCl_2$, the inhibitory effect of Pb^{2+} on PS II was also noticed, since the variable fluorescence was quenched when Pb^{2+} concentration had exceeded 0.2 mM and reached a steady state level at 2 mM Pb^{2+}. With the absence of $CaCl_2$, 0.1 mM Pb^{2+} was enough to quench the variable fluorescence to a steady state level. The Pb^{2+} inhibition of WSC did not significantly decrease the PS II photochemistry, since in the presence of 0.5 mM DPC, the variable fluorescence yield was preserved by 80% (Fig. 5). These results provide a strong evidence that Pb^{2+} inhibitory site is located at WSC. The part of variable fluorescence which was not quenched by Pb^{2+} probably belonged to PS II not involved in the electron-transport phenomena [13].

In the water-splitting complex, the inorganic cofactors, Ca^{2+} and Cl^-, are associated with process of water oxidation [3]. Binding of these two ions in their respective sites is essential in order to maintain the structure, function and integrity of the WSC [14]. Since our results show that Pb^{2+} inhibition of PS II activity can be prevented by the presence of $CaCl_2$, we interpret that Pb^{2+} competes for binding to the Ca^{2+} and Cl^- active sites in the vicinity of WSC. This interpretation is supported by the observation of $CaCl_2$ when added along with Pb^{2+} in protein-depleted PS II, a fairly competitive interaction between Pb^{2+} and $CaCl_2$ was obtained. In this reaction, Pb^{2+} may replace Ca^{2+} and Cl^- from their native sites in WSC and consequently inhibits oxygen evolution. However, the binding possibilities of Pb^{2+} become restricted when exogenous $CaCl_2$ was added and this consequently resulted in $CaCl_2$ protection against Pb^{2+} inhibition. We observed that adding $CaCl_2$ could not restore PS II activity which was previously inhibited by Pb^{2+}. This suggests a stronger binding affinity of Pb^{2+} than Ca^{2+} and Cl^- for the active sites in WSC. A similar effect was earlier seen in the study of lanthanide interaction with Ca^{2+} function in WSC [2]. It appears that the presence of Pb^{2+} may deplete the WSC from endogenous Ca^{2+} and Cl^- through competitive binding interaction and a higher binding affinity. Our results show that $CaCl_2$ failed to protect PS II from Pb^{2+} inhibition when Pb^{2+} concentration exceeded 2 mM. Therefore, it suggests that this type of inhibition may induce an irreversible modification of WSC. Since DPC can restore the activity, it indicates that Pb^{2+}-induced irreversible

Table I

Effects of additives on the PS II electron-transport activity ($H_2O \rightarrow$ DCIP) in the intact PS II membranes in the absence and presence of $Pb(NO_3)_2$ (0.2 mM)

Additions	DCIP photoreduction (μmol/mg Chl/h)		
	Without $Pb(NO_3)_2$	With $Pb(NO_3)_2$	Percentage of inhibition
None	199	0	100
$CaCl_2$	228	171	25
$Ca(NO_3)_2$	190	120	37
NaCl	215	150	30
$NaNO_3$	171	12	93
$MnCl_2$	160	106	33
$MnSO_4$	106	0	100
$MgCl_2$	171	97	43
$Mg(NO_3)_2$	150	6	96

Additive concentrations: 15 mM

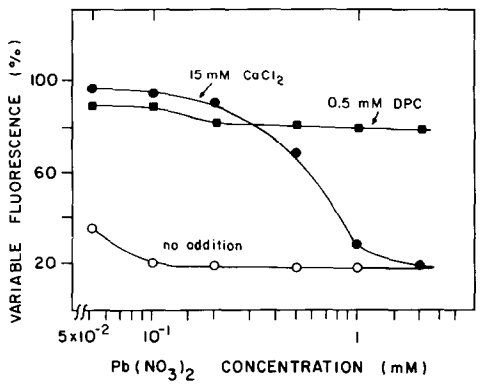

Fig. 5. Quenching of variable fluorescence by $Pb(NO_3)_2$ in intact PS II membranes. No addition (○); $CaCl_2$ (15 mM) (●); DPC (0.5 mM) (■).

modification of WSC may be mediated via not only the loss of Ca^{2+} and Cl^-, but also by the destruction of manganese active sites. This is consistent with previous reports showing that DPC is only an efficient electron donor to PS II when WSC is depleted in manganese [15–17].

In conclusion, our results show that Pb^{2+} induced an inhibition on the oxidizing site of PS II located before the electron donation site of DPC. The competitive interaction of Pb^{2+} with $CaCl_2$ is a supportive evidence that this inhibition is associated with the function of WSC, which is dependent on Ca^{2+} and Cl^- role. Therefore, in WSC, Pb^{2+} competes for binding to Ca^{2+}- and Cl^--active sites, induces a release of these cofactors and consequently inhibits the PS II activity.

Acknowledgements: This work was supported by the National Science and Engineering Research Council (NSERC) of Canada through the Grants No. G-1929, G-1930 and A-3047 given to R.P.

REFERENCES

[1] Miles, C.D., Brandle, J.R., Daniel, D.J., Chu-Der, O., Schnare, P.D. and Uhlik, D.J. (1972) Plant Physiol. 49, 820–825.
[2] Ghanotakis, D.F., Babcock, G.T. and Yocum, C.F. (1985) Biochim. Biophys. Acta 809, 173–180.
[3] Homann, P.H. (1988) Photosynth. Res. 15, 205–220.
[4] Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–236.
[5] Rashid, A. and Carpentier, R. (1989) FEBS Lett. 258, 331–334.
[6] Nakatani, H.Y. (1984) Biochem. Biophys. Res. Commun. 120, 299–304.
[7] Popovic, R.B., Kyle, D.J., Cohen, A.S. and Zalik, S. (1979) Plant Physiol. 64, 721–726.
[8] Morissette, J.C. and Popovic, R. (1987) Biochem. Biophys. Res. Commun. 149, 385–390.
[9] Rashid, A. and Carpentier, R. (1990) Photosynth. Res. 24, 221–227.
[10] Ghanotakis, D.F., Babcock, G.T. and Yocum, C.F. (1984) FEBS Lett. 167, 127–130.
[11] Samson, G., Morissette, J.C. and Popovic, R. (1988) Photochem. Photobiol. 48, 329–332.
[12] Mohanty, N., Vass, I. and Demeter, S. (1989) Physiol. Plant. 76, 386–390.
[13] Renganathan, M. and Bose, S. (1989) Biochim. Biophys. Acta 974, 247–253.
[14] Coleman, W.J. (1990) Photosynth. Res. 23, 1–27.
[15] Kuwabara, T. and Murata, N. (1982) Plant Cell Physiol. 23, 533–539.
[16] Inoue, H. and Wada, T. (1987) Plant Cell Physiol. 28, 767–773.
[17] Hsu, B.D., Lee, J.Y. and Pan, R.L. (1987) Biochim. Biophys. Acta 890, 89–96.