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Alteration of the structure and function of photosystem I by Pb^{2+}

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ABSTRACT

The toxic effects of Pb^{2+} on photosynthetic electron transport were studied in photosystem I (PSI) submembrane fractions isolated from spinach. Structural and spectroscopic analysis using FTIR, fluorescence and X-ray photoelectron spectroscopy (XPS) showed that Pb^{2+} binds with proteins *via* oxygen and nitrogen atoms with an overall binding constant of $K_{Pb\text{-}PSI} = 4.9 \times 10^3$ (±0.2) M^{-1} and the number of bound Pb^{2+} cation was 0.9 per PSI complex. Pb^{2+} binding altered the protein conformation indicating a partial protein destabilization. Electron transport and P700 photooxidation/reduction measurements showed that the interaction of Pb^{2+} cations with PSI produced a donor side limitation of electron transport presumably due to Pb^{2+} binding to or in the vicinity of plastocyanin.

1. Introduction

Lead is a potent environmental toxin that, as a result of human activity, has accumulated 1000-fold above its natural level of average10 ppm with a range of 7–20 ppm present in surface agricultural soils across the United States [1,2]. Plants exposed to toxic metal cations need to develop some degree of tolerance to metal toxicity in order to survive because the photosynthetic organisms are highly sensitive to it [3]. While the noxious effects of lead on plant growth have been known for many years, the exact mechanism of Pb^{2+} toxicity still eludes the scientific community. Heavy metals in general can cause the disruption of many physiological functions and substitute for the essential ions as well (for reviews see [4–6]). Indeed, it is well established that at high internal concentrations, Pb disturbs almost all physiological processes in plants.

Photosynthesis is one of the most Pb-sensitive processes in plants. Lead toxicity has multifunctional adverse effects on photosynthetic CO_2 fixation *in vivo* and *in vitro*. Long term exposure results in reduced leaf growth, decreased level of photosynthetic pigments, altered chloroplasts ultra-structure and decreased enzymatic activity of CO_2 assimilation [7–9]. *In vitro*, with isolated chloroplasts, the inhibition of photosynthetic electron transport and photochemical activity by Pb^{2+} has been observed [10–13]. However, its mode of action on the photosynthetic apparatus is not fully understood. Although some knowledge has been accumulated about the interaction of lead with PSII where it is reported that Pb^{2+} mainly affects the oxygen evolving complex (see [13] and references therein), very little information is available with regard to the action of Pb^{2+} on PSI.

PSI is a pigment-protein complex located in the thylakoid membrane of cyanobacteria, algae and chloroplasts of higher plants that mediates electron flow from PSII to $NADP^+$. The excitation energy in the antenna pigments of PSI is promptly transferred to P700 (a special pair of Chl *a* molecules and the primary electron donor in PSI). In its lowest electronically excited state, P700 donates an electron to the primary electron acceptor, A0 (a Chl *a* molecule). Charge separation and stabilization through the secondary acceptor A1 (a phylloquinone molecule, the secondary electron acceptor), and terminal iron–sulfur [4Fe–4S] centers (FX, FA, and FB), result in the reduction of ferredoxin (Fd) located at the stromal side [14,15]. The $P700^+$ cation formed after charge separation is then re-reduced by the copper protein plastocyanin (PC), a mobile carrier of the thylakoids lumen. In turn, the oxidized PC receives electrons from Cyt b6/f. PSII photooxidizes water to oxygen and supplies electrons to Cyt b6/f [14,16–18].

In this work, we have examined the interaction of Pb^{2+} with PSI in isolated PSI submembrane fractions to determine the Pb^{2+} binding site and the effect of metal ion interaction on the photosystem. Various spectroscopic approaches such as FTIR, XPS and fluorescence were applied along with measurements of oxygen uptake and P700 photoxidation/reduction kinetics to assess the effect of Pb^{2+} on PSI structure and function. It was found that Pb-protein interaction greatly influenced the stability and conformation of PSI leading to the inhibition of the electron transfer process on the donor side of PSI. To our knowledge, this is the first study showing a possible inhibitory site of Pb^{2+} on PC.

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2. Materials and methods

2.1. Isolation of PSI submembrane fractions

PSI submembrane fractions were isolated from fresh spinach leaves obtained from the local market, according to the procedure reported [19] with some modifications [20]. The isolated preparations with Chl content of 2–3 mg Chl mL^{-1} were suspended in a medium containing 20 mM Tricine-KOH buffer (pH 7.8), 10 mM NaCl, 10 mM KCl, and 5 mM $MgCl_2$, and stored at −80 °C until use. Chl concentration was determined in 80% acetone according to the published method [21]. The Chl a/b ratio was greater than 6.

2.2. FTIR spectroscopic measurements

Infrared spectra were recorded on a FTIR spectrometer (Impact 420 model), equipped with deuterated triglycine sulphate (DTGS) detector and KBr beam splitter using AgBr windows. $PbCl_2$ was added to the PSI submembrane fractions to obtain the desired Pb^{2+} concentrations of 0.125 and 0.5 mM. Spectra were collected after 4-h incubation of PSI with Pb at room temperature using hydrated films. Interferograms were accumulated over the spectral range 4000–600 cm^{-1} with a nominal resolution of 2 cm^{-1} and 100 scans. The difference spectra [(PSI submembrane fractions + Pb) – (PSI submembrane fractions)] were generated using the water combination mode at around 2300 cm^{-1} as standard [22]. When producing difference spectra, this band was adjusted to the baseline level in order to normalize the difference spectra.

2.3. Analysis of protein conformation

The analysis of the secondary structure of the proteins in the PSI submembrane fractions and their Pb^{2+} complexes was carried out on the basis of the procedure previously reported [23]. The protein secondary structure was determined from the shape of the amide I band located around 1650–1660 cm^{-1}. The FTIR spectra were smoothed and their baselines were corrected automatically using Grams AI software. Thus the root-mean square (rms) noise of every spectrum was calculated. By means of the second derivative in the spectral region 1700–1600 cm^{-1}, six major peaks were resolved. The above spectral region was deconvoluted by the curve-fitting method with the Levenberg-Marquadt algorithm and the peaks, corresponding to α-helix (1660–1654 cm^{-1}), β-sheet (1637–1614 cm^{-1}), turn (1678–1670 cm^{-1}), random coil (1648–1638 cm^{-1}) and β-antiparallel (1691–1680 cm^{-1}), were adjusted and the area was measured with the Gaussian function. The area of all the component bands assigned to a given conformation were then summed up and divided by the total area [24,25]. The curve-fitting analysis was performed using the GRAMS/AI Version 7.01 software of the Galactic Industries Corporation.

2.4. X-ray photoelectron spectroscopy

XPS was performed on a Kratos Axis Ultra spectrometer (Kratos Analytical Ltd., UK), using a monochromatic Al Ka X-ray source (E = 1486.6 eV) with a power of 225 W at a take-off angle of 90° relative to the sample surface. 250 μL of the sample which consisted of PSI submembrane fractions or PSI submembrane fractions mixed with $PbCl_2$ (500 μM) were dropped on an aluminum substrate and dried in a vacuum desiccator overnight to obtain a thin film. The dried sample was then transferred to the XPS sample holder. The measurements were made under a high vacuum of 10^{-9} torr at room temperature. The surface of the sample was 20 mm^2 and the investigated area was typically 1 × 2 mm^2. Survey spectra for each sample over a binding energy range of 0–1300 eV were an average of three scans (at three different points) acquired at a pass energy of 160 eV and resolution of 1 eV/step (lens in hybrid mode, which assures maximum sensitivity). High-resolution spectra of C 1s, N 1s and O 1s were an average of five scans acquired at a pass energy of 40 eV and resolution of 0.1 eV/step for quantitative measurements of binding energy and atomic concentration. Because of the potential degradation of the surface during X-ray exposure, the spectra were collected in the same order (survey, C 1s, O 1s, N 1s) such that the amount of exposure to X-rays was equivalent for all analyzed samples. The CasaXPS software was used for background subtraction (Shirley-type), peak integration, fitting and quantitative chemical analysis. The C 1s (C–C) peak at 285 eV was used to calibrate the binding energy scale. Binding energies values are given at ±0.2 eV. Gaussian peak profiles were used for spectral deconvolution of all spectra as described before [26]. The assay medium contained 20 mM Tricine-KOH buffer (pH 7.8), 10 mM NaCl, 10 mM KCl, and 5 mM $MgCl_2$, and a final chlorophyll concentration of 250 μg mL^{-1}. The magnesium metal binding energy was not recorded due to the poor signal to background counting ratio caused by the low core level cross-section of the Mg 1s level, as observed in other report [27].

2.5. Fluorescence spectroscopy

Fluorometric experiments were carried out at room temperature (24.5 ± 0.5 °C) with a Perkin Elmer LS55 Spectrometer equipped with a red-sensitive photomultiplier R928. Photosynthetic samples were kept in the dark before use. PSI submembrane fractions were diluted to 5 μg mL^{-1} with the buffer. PSI samples were excited at 436 nm and fluorescence emission spectra were recorded from 600 to 800 nm as previously reported [28]. The excitation and emission slit widths were fixed at 5 and 2.5 nm, respectively, and emission spectra were corrected according to the photomultiplier sensitivity using the correction factor spectrum provided by Perkin-Elmer.

2.6. Oxygen uptake measurements

PSI-mediated electron transfer from reduced 2,6-dichlorophenolindophenol ($DCPIPH_2$) to methylviologen (MV) was measured by light-induced O_2 consumption using a Clark type oxygen electrode at 24 °C as described earlier [29]. White light was provided by a 150 W quartz-halogen projector lamp. The samples were diluted in a buffer containing 20 mM Tricine-KOH (pH 7.8), 10 mM NaCl, 10 mM KCl, 5 mM $MgCl_2$, 500 μM MV, 1 mM NaN_3, 100 μM DCIP (or 50 μM TMPD), 1 mM Asc, and 50 μM DCMU to obtain PS I submembrane fractions with 10 μg Chl mL^{-1}. Prior to the measurements, the samples were incubated in dark for 5 min.

2.7. Redox state of P700

Photo-oxidation and subsequent dark reduction measurements of P700 were monitored as light-induced absorbance changes at 830 nm, (ΔA_{830}), at room temperature, using the dual wavelength emitter detector ED-P700DW connected via a PAM-101 fluorometer (Walz, Effeltrich, Germany). The ED-P700DW unit strictly detects the differential absorbance changes at 830 nm (ΔA_{830}) ascribed to the absorption due to $P700^+$ cation radical and removes the plastocyanin absorbance changes as described before [30,31]. Photon flux density of far-red (FR) light (75 μmol m^{-2} s^{-1}) was obtained by passing the beam from a Filtre-Lite light source (Microview, Thornhill, ON, Canada) through a RG-9 filter (Schott, Mainz, Germany). The assay medium contained 20 mM Tricine-KOH buffer (pH 7.8), 10 mM NaCl, 10 mM KCl, and 5 mM $MgCl_2$, and a final Chl concentration of 200 μg mL^{-1}.