

## Effects of $Pb^{2+}$ on the Structure and Function of Photosystem II of *Spirodela polyrrhiza*

Ling Qufei · Hong Fashui

Received: 8 September 2008 / Accepted: 14 November 2008 /  
Published online: 4 December 2008  
© Humana Press Inc. 2008

**Abstract** Lead ( $Pb^{2+}$ ) is a well-known highly toxic element, and *Spirodela polyrrhiza* is one of the hydrophytes most sensitive to  $Pb^{2+}$ , which might be highly toxic to photosynthesis. However, the mechanism by which  $Pb^{2+}$  inhibits energy transfer and conversion efficiency remains unclear. Here, we report the effects of  $Pb^{2+}$  on the secondary structure and function of photosystem II (PS II) of *S. polyrrhiza* using spectral methods. We found that  $Pb^{2+}$  accumulated in PS II and damaged its secondary structure, decreased the absorbance of visible light, inhibited energy transfer among amino acids within the PS II protein–pigment complex, and reduced energy transport from tyrosine residue to chlorophyll a. Taken together,  $Pb^{2+}$  exposure damaged the structure and function of *S. polyrrhiza* PS II.

**Keywords**  $Pb^{2+}$  · *Spirodela polyrrhiza* · Photosystem II · Energy transfer · Photochemical activity · Oxygen evolving rate

### Introduction

Toxic levels of heavy metals, such as lead (Pb), occur in natural and agricultural systems as a result of environmental pollution from mining, smelting, manufacturing, agricultural, and waste disposal technologies (1), and there has been much interest in the toxic effects of heavy metals on plants (2–7). *Spirodela polyrrhiza* is a common species of duckweed in China; it has a larger leaf area and more roots than other duckweed species (8). It is also a species that is used in wastewater treatment systems and is one of the hydrophytes most sensitive to  $Pb^{2+}$ . Furthermore, photosynthesis is one of the most  $Pb^{2+}$ -sensitive processes in plants (9). The effects of  $Pb^{2+}$  are multifactorial, affecting both in vivo and in vitro photosynthetic  $CO_2$  fixation. Long-term exposure results in reduced leaf growth, decreased levels of photosynthetic pigments, altered chloroplast structure, and decreased enzyme activities for  $CO_2$  assimilation (10, 11). The total chlorophyll content and relative

---

L. Qufei · H. Fashui (✉)  
Medical College of Suzhou University, Suzhou 215123, People's Republic of China  
e-mail: Hongfsh\_cn@sina.com

proportions of chlorophylls a and b are reduced through an inhibition of chlorophyll biosynthesis (12–16). The substitution of the central atom of chlorophyll, magnesium, by lead in vivo prevents photosynthetic light-harvesting in affected chlorophyll molecules, resulting in a breakdown of photosynthesis (17). Lead also significantly inhibits the Hill reaction activity of spinach chloroplast in addition to photophosphorylation, and lead has a more conspicuous effect on cyclic photophosphorylation than on noncyclic photophosphorylation (18). In vitro studies with isolated chloroplasts have shown that Pb inhibits photosynthetic electron transport and the photochemical activity of photosystem II (PS II) (9, 19); however, whether or not Pb inhibits energy transfer among amino acids within the PS II protein complex and from amino acid residues to chlorophyll a remains unclear.

PS II is located at the thylakoid membrane of the chloroplast, and its protein complex executes the PSII function. In the present study, we isolated and purified PS II particles from *S. polyrrhiza* exposed to different concentrations of PbCl<sub>2</sub>. We studied the effects of Pb<sup>2+</sup> on secondary structure, energy transfer among amino acids and from amino acid residues to chlorophyll a in the PS II protein–pigment complex, as well as its impact on PS II photochemical activity, using various spectral methods.

## Materials and Methods

### Reagents

Triton-X-100, 2- (*N*-morpholino) ethanesulfonic acid (MES) were purchased from Sigma. Other reagents were acquired in China and were of analytical grade.

### Material Treatment and Culture

The *S. polyrrhiza* used in this study were collected from a wetland near Tai Lake (located in Suzhou City, Jiang Province, China). The experiments were conducted under laboratory conditions with fluorescent lamps with a light intensity of 500  $\mu\text{M}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (16 h light, 8 h dark). The temperature was 25°C. The growth medium used to culture duckweed was based on the Hoagland's culture media (20). Cultivation of *S. polyrrhiza* was performed in 500-mL plastic breakers using 400 mL of Hoagland's medium supplemented with 0, 25, 50, or 100  $\mu\text{M}$  PbCl<sub>2</sub>. Forty healthy fronds of *S. polyrrhiza* were placed in the plastic containers at the beginning of each experiment. Hoagland medium was replaced once every 7 days so as to avoid nutrient exhaustion due to algal growth. The pH of each culture was always maintained at about 7 (6.5–7.5) in all experiments by the addition of 1 M HCl or KOH every day. Loss of water by evaporation was compensated for by the addition of demineralized water. *Spirodela polyrrhiza* was exposed to PbCl<sub>2</sub> + Hoagland's medium for 35 days, and each experiment had five replicates.

### PS II Preparation

We prepared PS II particles from *S. polyrrhiza* using the method of Kuwabara and Murata et al. (21). Chloroplasts were extracted from *S. polyrrhiza* leaves with a medium containing 100 mM sucrose, 200 mM NaCl, and 50 mM K phosphate buffer (pH 7.4). The chloroplasts were then suspended in a second medium containing 300 mM sucrose, 50 M NaCl, and 50 mM K phosphate buffer (pH 6.9) at a chlorophyll concentration of

2–3 mg/mL. An aqueous solution of Triton X-100 (20% W/V) was added to the suspension with stirring until the ratio of Triton X-100 to chlorophyll became 25:1 (W/W). After incubation for 1 min, the suspension was centrifuged at 1,000×g for 2 min; the pellet was discarded, and the supernatant was centrifuged at 35,000×g for 10 min. The resultant pellet was collected and resuspended in 40 mM K phosphate buffer (pH 6.9), and the suspension was again centrifuged at 1,000×g for 2 min. The pellet was discarded, and the supernatant centrifuged at 35,000×g for 10 min. The final pellet constituted the preparation of PS II particles used in experiments. The ratio of chlorophylls a to b in the preparation was about 2.0:1. After incubating for 15 min in dim light, the PS II particles were collected by centrifugation at 144,000×g for 30 min. All of the above procedures were performed at 0–4°C. Chlorophyll of PS II was extracted in chilled 80% acetone and estimated spectrophotometrically (22).

### Pb<sup>2+</sup> Content of PS II

About 0.5 mg of PS II particles were digested and analyzed for Pb<sup>2+</sup> content. Briefly, prior to elemental analysis, the PS II particles were digested overnight in nitric acid (ultrapure grade). After adding PS II particles were digested in nitric acid (ultrapure grade) overnight. After adding 0.5 mL of H<sub>2</sub>O<sub>2</sub>, the mixed solutions were heated to about 160°C using a high-pressure reaction container in an oven chamber until the samples were completely digested. The solutions were then heated to 120°C until the solutions were colorless and clear to remove remaining nitric acid. Finally, the solutions were diluted to 4 mL with 2% nitric acid. Inductively coupled plasma-mass spectrometry (ICP-MS, POEMS, Thermo Jarrel Ash, USA) was used to analyze the Pb<sup>2+</sup> concentration in the samples. Data are expressed as nanograms per milligram PS II particles.

### Circular Dichroism (CD) Spectrum of the Purified PS II

The CD spectra of the purified PSII were analyzed at room temperature on a JASCO-J-810 spectropolarimeter with a quartz sample cell with an optical path length of 1 cm. The concentration of PS II was 0.2 mg·mL<sup>-1</sup>. Molecular ellipticities [θ] in degrees per square centimeter per decimole were calculated using a mean residue weight of 115. The secondary structure indexes, α-helix, β-sheet, β-turn, and random coil of the PS II samples were determined by Perczel's method (23). The method, called "convex constraint analysis," is a general deconvolution method for a CD spectra set of any variety of conformational type. The algorithm, which is based on a set of three constraints, is able to deconvolute a set of CD curves to its common "pure"-component curves and conformational weights. To analyze a single CD spectrum with this method, the spectrum is appended to the data set used as a reference data set.

### Absorption Spectrum and Fluorescence Spectrum of the Purified PS II

The absorption spectrum of the purified PS II particles was measured from 400–750 nm at room temperature with a dual-beam spectrophotometer (UV-3010, Hitachi, Japan). The fluorescence excitation spectra of the purified particles were recorded at 273 K from 200 to 300 nm when emission was at 304 nm, and from 200 to 400 nm when emission was at 683 nm, using a F-4500 fluorometer (Hitachi, Japan). The fluorescence emission was recorded at 273 K from 290 to 400 nm when excitation was at 280 nm and from 600 to 800 nm when excitation was at 436 nm.

## Activities of the Oxygen Evolution of the Purified PSII

The PS II particles were suspended at 25°C in a medium containing 300 mM sucrose, 10 mM NaCl, 0.05% bovine serum albumin, and 25 mM MES-NaOH (pH 6.5). The concentration of the PS II particles was equivalent to about 15 µg of chlorophyll per milliliters. The oxygen evolution was measured with an Oxygraph oxygen electrode (Hansatech instruments, UK). All experiments were independently performed, and the presented data represent the average of the recordings from five independent experiments.

## Statistical Analysis

Data were analyzed with analysis of variance, and the statistical significance of the differences between treatment means was determined using the Scheffe test ( $p < 0.05$ ).

## Results

### Pb<sup>2+</sup> Content in PS II

The Pb<sup>2+</sup> contents of *S. polyrrhiza* PSII are presented in Table 1, which suggests that the Pb<sup>2+</sup> content of PSII from Pb<sup>2+</sup>-treated *S. polyrrhiza* increased with increasing Pb<sup>2+</sup> exposure concentration; no Pb<sup>2+</sup> was detected in the control.

### The Secondary Structure of PS II

The CD spectra of the PS II particles of *S. polyrrhiza* are shown in Fig. 1, which indicates that the CD spectra of the PSII particles from Pb<sup>2+</sup>-treated *S. polyrrhiza* are the same as that of the control, which are negative Cotton peaks in 208 and 220 nm, the α-helix and β-sheet contents of PS II were decreased, and the β-turn and random coil contents were increased in the PSII preparations from various Pb<sup>2+</sup>-treated *S. polyrrhiza* (Table 2).

### Light Absorption of PS II

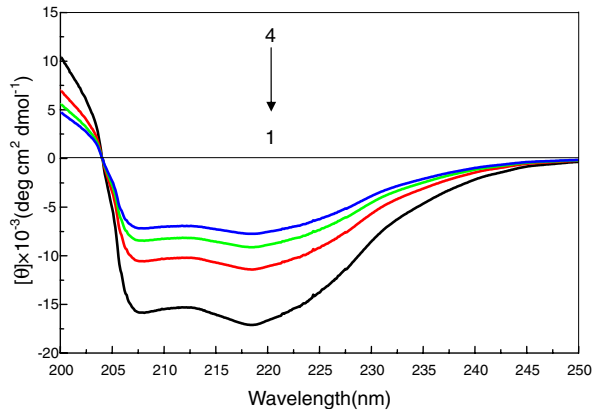
The visible absorption spectrum of the PS II particles purified from *S. polyrrhiza* is shown in Fig. 2. Chlorophyll-a (chl<sub>a</sub>) absorption peaks in the red region and blue region were observed at 660–670 nm and at 430 nm (curve 1), respectively; there was a distinct shoulder at 640–650 nm, which can be ascribed to chlorophyll-b (chl<sub>b</sub>) absorption. A wide shoulder at around 450–470 nm indicates the presence of chl<sub>b</sub> and carotenoid. The absorption peak at about 415 nm suggests the presence cytochrome b-559 hem and pheophytin in the complex. These results are in agreement with those of previous reports

**Table 1** Effect of Pb<sup>2+</sup> Treatment on Pb<sup>2+</sup> Content Binding to PSII Particles of *S. polyrrhiza*

Pb <sup>2+</sup> (µM)	Control (No Pb)	25	50	100
Pb <sup>2+</sup> content (ng/g PS II particles)	Not detected	80.75±4.04	128.31±6.42*	172.49±8.62*

Data values are mean ± standard error of five experimental replicates. Cells marked with stars are significantly different from 25-µmol/L Pb<sup>2+</sup> treatment at the 5% confidence level

**Fig. 1** Effects of Pb<sup>2+</sup> on CD spectra of PS II of *S. polyrrhiza*. CD spectra were recorded at room temperature. The concentration of PS II was 0.2 mg/mL. (1) Control; (2) 25  $\mu$ M; (3) 50  $\mu$ M; (4) 100  $\mu$ M



(23–27). Lines 2–4 in Fig. 2 show that the visible absorption spectra of the PS II particles from various PbCl<sub>2</sub>-treated *S. polyrrhiza* were nearly the same as that of the control; however, the absorption intensities were lower than that of the control.

#### Energy Transfer Among Amino Acids Within PS II

The fluorescence emission was recorded from 290 to 400 nm at 273 K. The emission peak of PS II at 304 nm was observed when excitation was at 280 nm, but there was no emission peak from 325 to 350 nm (Fig. 3a). In excitation spectra of PS II, the excitation peaks of PS II at 230 and 277 nm were observed when emission was at 304 nm, respectively (Fig. 3b). The excitation peak at 277 nm corresponds to absorption peak of tyrosine residues, suggesting that the emission peak at 304 nm was attributed to tyrosine residues of PS II. The carrier protein of pigment–protein complex of PS II displayed a fluorescence characteristic of tyrosine and was attributed to A type of protein (28). In addition, Fig. 3b shows the excitation peak of PS II at 230 nm, showing that excited energy from amino acids at 230 nm could be transferred to tyrosine group, which resulted in fluorescence emission.

Figure 3a,b shows that PbCl<sub>2</sub> treatments at various concentrations had great influence on fluorescence spectra of PSII particles, i.e., the emission peak intensity at 304 nm was lower than that of the control; the excitation peaks of amino acids at 230 nm and tyrosine residues reduced, respectively, and the excitation peak at 230 nm was red-shifted by 2 nm and the ratio of F<sub>278</sub>/F<sub>230</sub> decreased.

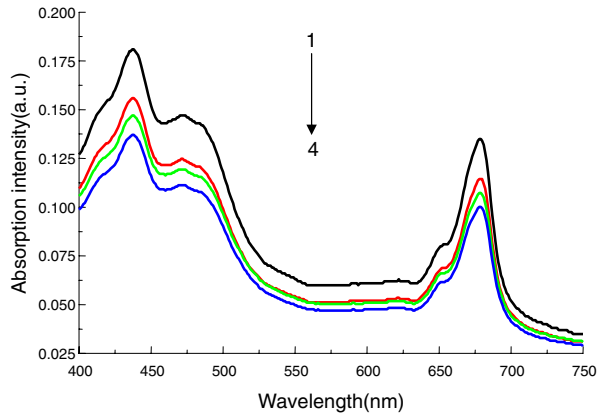
#### Energy Transfer Between Protein and Chlorophyll-a of PS II

The fluorescence excitation spectra of chl a of PS II complex when emission was at 683 nm are shown in Fig. 4. The excitation peak at 343 nm is attributed to chl a, the peaks at 230

**Table 2** Effect of Pb<sup>2+</sup> on Secondary Structure of PS II of *S. polyrrhiza*

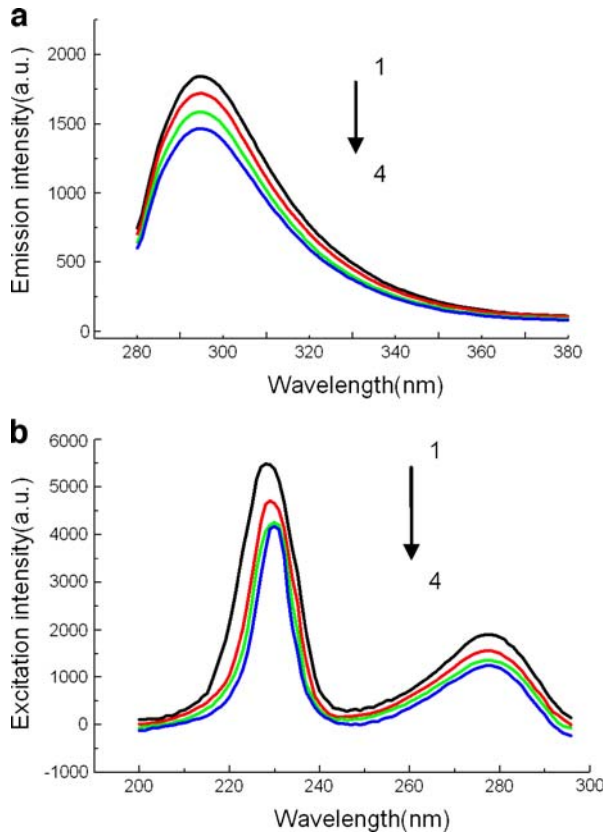
Pb <sup>2+</sup> ( $\mu$ M)	$\alpha$ -helix	$\beta$ -sheet	$\beta$ -turn	Random coil
Control (no Pb)	50.73%	38.02%	7.3%	3.94%
25	19.89%	35.95%	33.76%	10.4%
50	18.61%	34.99%	31.19%	15.2%
100	17.7%	23.76%	42.21%	16.33%

**Fig. 2** Effect of  $\text{Pb}^{2+}$  on visible absorption spectra of PS II particles at room temperature. (1) Control; (2) 25  $\mu\text{M}$ ; (3) 50  $\mu\text{M}$ ; (4) 100  $\mu\text{M}$

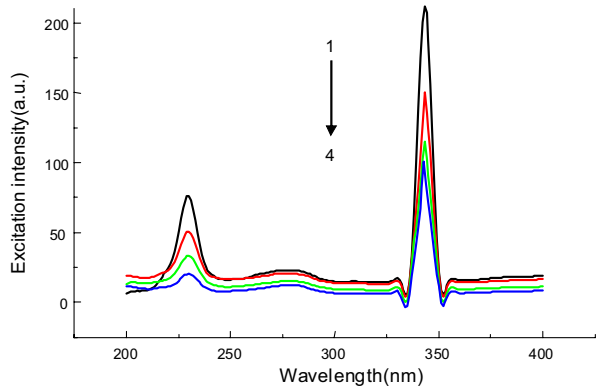


and 277 nm are consistent with excitation peaks of intrinsic fluorescence of protein when emission was at 304 nm. It shows that there was an energy transfer between protein and chl *a* within the pigment–protein complex, i.e., the excitation energy from amino acids at 230 nm and tyrosine residues at 278 nm could be transferred to chl *a*. Upon  $\text{PbCl}_2$  treatment, the obvious reduction of excitation peaks at 230, 278, and 343 nm can be seen in Fig. 4; the reduction at 343 nm was the most significant.

**Fig. 3** Effect of  $\text{Pb}^{2+}$  on fluorescence spectra of PS II particles at 273 K. **a** Excitation at 280 nm; **b** emission at 304 nm. (1) Control; (2) 25  $\mu\text{M}$ ; (3) 50  $\mu\text{M}$ ; (4) 100  $\mu\text{M}$



**Fig. 4** Effect of Pb<sup>2+</sup> on fluorescence excitation spectra of chlorophyll a of PSII particles at 273 K. Emission at 683 nm. (1) Control; (2) 25  $\mu$ M; (3) 50  $\mu$ M; (4) 100  $\mu$ M



### Fluorescence Quantum Yield of PS II

In Fig. 5, the fluorescence emission spectrum at 273 K exhibits a main peak at 683 nm, which is a characteristic of the PSII particles when the excitation is 436 nm. The major part of the emission observed at 683 nm originated from the charge recombination of P680<sup>+</sup> and the reduced pheophytin (29, 30). However, the emission peaks treated with various concentration of PbCl<sub>2</sub> (lines 2–4) were blue-shifted by 2 nm, and the peak intensities (fluorescence quantum yield) were decreased by 35.61%, 40.44%, and 45.80%, respectively.

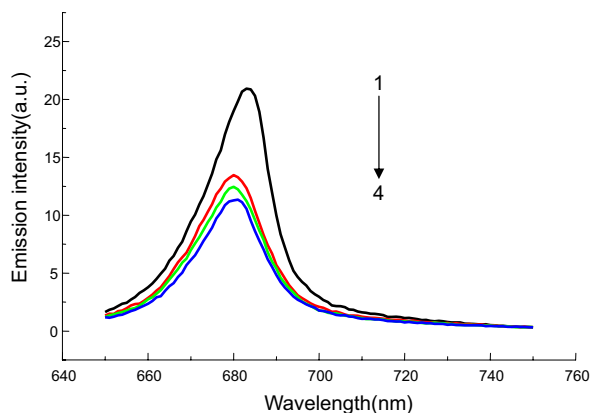
### The Oxygen Evolution of the PS II

The effects of Pb<sup>2+</sup> on oxygen evolution of PS II particles are shown in Fig. 6. The O<sub>2</sub>-evolving rates treated with various concentration of PbCl<sub>2</sub> were lower by 41.04%, 53.64%, and 62.77%, respectively, compared with the control ( $p < 5\%$ ).

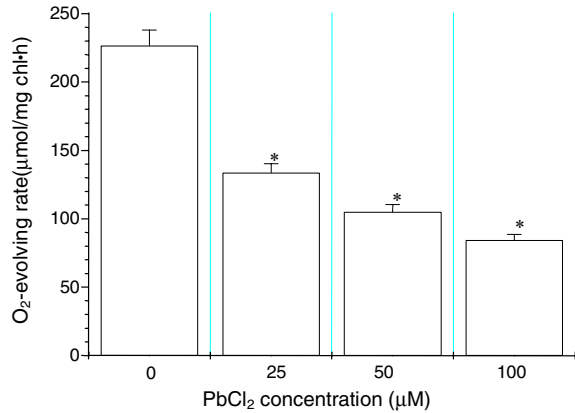
## Discussions

In order to determine whether Pb<sup>2+</sup> had entered chloroplasts and bound to PS II, we measured the Pb<sup>2+</sup> content of PS II from the four PbCl<sub>2</sub>-treated groups of *S. polyrrhiza*. We

**Fig. 5** Effect of Pb<sup>2+</sup> on fluorescence emission spectra of PS II particles at 273 K. Excitation at 280 nm. (1) Control; (2) 25  $\mu$ M; (3) 50  $\mu$ M; (4) 100  $\mu$ M



**Fig. 6** Effect of  $\text{Pb}^{2+}$  on oxygen evolution of PS II particles. Bars marked with a *star* were different from the other bars in the panel at the 5% significant level. *Bars* indicate mean and *error bars* are standard errors



found that  $\text{Pb}^{2+}$  accumulated in the PSII of *S. polyrrhiza* and also that  $\text{Pb}^{2+}$  increased with increasing  $\text{Pb}^{2+}$  exposure; the control was not detected. CD spectra suggested that the secondary structure of PS II from various  $\text{Pb}^{2+}$ -treated *S. polyrrhiza* was damaged, thus establishing that  $\text{Pb}^{2+}$  was indeed bound to the PS II.

Experimental research has shown that the visible light absorption of PS II is obviously decreased in  $\text{PbCl}_2$ -treated *S. polyrrhiza*. This is because  $\text{PbCl}_2$  destroyed the conformation of PS II, and the total chlorophyll content and relative contents proportion of chl a and chl b were reduced through the inhibition of chlorophyll biosynthesis (11–15). The substitution of the central atom of chlorophyll, magnesium, by lead in vivo prevents photosynthetic light-harvesting in the affected chlorophyll molecules, resulting in a reduction of light absorbance (16, 18, 19).

We demonstrate that PS II exhibited a fluorescence characteristics of tyrosine; there is an energy transfer between amino acids at 230 nm and tyrosine at 278 nm and between protein and chlorophyll-a of intrinsic protein complex of PS II. The absorption of PSII at 230 nm might be attributable to polypeptide, or to cysteine residues of protein, which have an absorption peak at 235 nm (28, 31). The cysteine residue for the intrinsic carrier protein of PS II could absorb light and be excited. Its excitation energy was transferred to a tyrosine group and caused the enhancement of excitation energy at 277 nm, whereas it leads to an increase of excitation energy of chl a at 343 nm. The ratio of  $F_{278}/F_{230}$  of PS II from  $\text{PbCl}_2$ -treated *S. polyrrhiza* decreased, suggesting that  $\text{PbCl}_2$  inhibited the excitation energy transfer from cysteine residue to tyrosine residue within PS II complex. In contrast, the ratio of  $F_{278}/F_{343}$  was gradually increased by the  $\text{PbCl}_2$  treatments, indicating that  $\text{PbCl}_2$  could inhibit excitation energy transfer from cysteine residue to tyrosine residue, and from tyrosine residue to chl a within PS II complex; this is because the  $\text{Pb}^{2+}$  bond to PSII and the protein skeleton was impaired, and a rotation of protein and pigments on the thylakoid membrane was produced by  $\text{Pb}^{2+}$ . Therefore, the excitation energy from amino acids was barely transported to chl a, resulting in a reduction of the excitation intensity at 343 nm.

The reduction of fluorescence quantum yield of PS II reaction center pigment—chl a (P680)—was because the excitation energy from LHC II, CP43 and CP47 of the core antenna was inefficiently transferred to P680 upon  $\text{Pb}^{2+}$  treatment. We speculate that  $\text{Pb}^{2+}$  might impair the bound state of chl a (P680) on the PS II reaction center. The reduction of the fluorescence quantum yield of PS II suggests that  $\text{Pb}^{2+}$  decreased the utilization and conversion efficiency of light energy within PS II.



Treatment with various concentrations of PbCl<sub>2</sub> treatment resulted in a decrease in oxygen evolution, indicating that PbCl<sub>2</sub> impaired the energy transfer among various compositions of PS II, slowed the transformation from light energy to electron energy, and inhibited electron transport, leading to the inhibition of water splitting and oxygen evolution. In addition, PbCl<sub>2</sub> destroyed the structure of tyrosine residues within PS II and impaired the function of secondary electron donors, which are thought to be attributable to tyrosine residues of D1 protein in PS II (32). Harvesting electrons and protons for tyrosine residues from water was thereby reduced.

Taken together, Pb<sup>2+</sup> bound to the PS II, which might replace Mg<sup>2+</sup> in chlorophyll or Ca<sup>2+</sup> in the oxygen-evolving center, resulted in alteration of PS II structure and inhibited both energy transfer within PS II and oxygen evolution.

**Acknowledgments** This work was supported by the National Natural Science Foundation of China (grant no. 30470150, 30800068).

## References

1. Foy CD, Chaney RL, White MC (1978) The physiology of metal toxicity in plants. *Annu Rev Plant Physiol* 29:511–566
2. Reed RH, Gadd GM (1990) Metal tolerance in eukaryotic and prokaryotic algae. In: Shaw AJ (ed) *Heavy metal tolerance in plants: evolutionary aspects*. CRC, Boca Raton
3. De Filippis LF, Pallaghy CK (1992) Heavy metals: sources and biological effects. In: Rai LC, Gaur JP (eds) *Phycological perspectives of water pollution*. E. Schweizerbart Verlag, Stuttgart
4. Luo LX, Sun TY (1998) Effect of cadmium-surfactant combined pollution on physiological characteristic of wheat leaf. *Chin J Appl Ecol* 9(1):95–100, (in Chinese)
5. Qin TC, Wu YS, Wang HX, Li QR (1998) Effect of cadmium, lead and their interactions on the physiological and ecological characteristics of root system of *Brassica chinensis*. *Acta Ecol Sin* 18 (3):320–325, (in Chinese)
6. Luo LX, Sun TY (1998) Effect of cadmium stress on lipid peroxidation in wheat leaves. *China Environ Sci* 18(1):72–75, (in Chinese)
7. Zhou CF, Wu GR, Shi GX, Lu CM (2001) The role of antioxidant systems in Cu<sup>2+</sup> stress resistance in *Spirodela polyrrhiza*. *Acta Bot Sin* 43:389–394
8. Yan SZ (1983) *The illustrated handbook of high aquatic plant in China*. Science, Beijing, p 294
9. Miles CD, Brandle JR, Daniel DJ, Chu-Der O, Schnare PD, Uhlik DJ (1972) Inhibition of photosystem II in isolated chloroplasts by lead. *Plant Physiol* 49:820–825
10. Parys E, Romanowska E, Siedlecka M, Poskuta JW (1998) The effect of lead on photosynthesis and respiration in detached leaves and in mesophyll protoplasts of *Pisum sativum*. *Acta Physiol Plant* 20:313–322
11. Shearan IS, Singh R (1993) Effect of heavy metals on photosynthesis in higher plants. In: Abrol YP, Mohanty P, Govindjee (eds) *Photosynthesis: photoreactions to plant productivity*. Kluwer, Dordrecht, pp 451–468
12. Ernst WHO, Nielssen HGM, Ten Bookum WM (2000) Combination toxicology of metal- enriched soils: physiological responses of a Zn- and Cd-resistant ecotypes of *Silene vulgaris* on polymetallic soils. *Environ Exp Bot* 43:55–71
13. Sinha SK, Srivastava HS, Tripathi RD (1993) Influence of some growth regulators and cations on inhibition of chlorophyll biosynthesis by lead in maize. *Bull Environ Contam Toxicol* 51:241–246
14. Van Assche JA, Clijsters H (1990) Effects of heavy metals on enzyme activity in plants. *Plant Cell Environ* 13:195–206
15. Wu X, Liu C, Qu CX, Huang H, Liu XQ, Chen L, Su MY, Hong FS (2008) Influences of PbCl<sub>2</sub> on the nitrogen metabolism of spinach. *Biol Trace Element Res* 121:258–265
16. Wu X, Huang H, Liu XQ, Chen L, Liu C, Su MY, Hong FS (2008) Oxidative stress induced by lead in chloroplast of spinach. *Biol Trace Elem Res*. doi:10.1007/s12011-008-8195-7
17. Kupper H, Kupper F, Spiller M (1996) Environmental relevance of heavy metal substituted chlorophylls using the example of water plants. *J Exp Bot* 47:259–266

18. Wu X, Liu C, Qu CX, Huang H, Liu XQ, Chen L, Hong FS (2008) Effects of lead on activities of photochemical reaction and key enzymes of carbon assimilation in spinach chloroplast. *Biol Trace Elem Res*. doi:10.1007/s12011-008-8196-6
19. Wu X, Hong FS, Liu C, Su MY, Zheng L, Gao FQ, Yang F (2008) Effects of  $Pb^{2+}$  on energy distribution and photochemical activity of spinach chloroplast. *Spectrochim Acta Part A* 69:738–742
20. Meider H (1984) Class experiments in plant physiology. George Allen and Unwin, London, pp 72–74
21. Kuwabara T, Murata N (1982) Inactivation of photosynthetic oxygen evolution and concomitant release of three polypeptides in the photosystem II particles of spinach chloroplasts. *Plant Cell Physiol* 23: 533–539
22. Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. *Plant Physiol* 24:1–15
23. Perczel A, Park K, Fasman GD (1992) Analysis of the circular dichroism spectrum of proteins using the convex constraint algorithm: A practical guide. *Anal Biochem* 203:83–93
24. Du LF, Tang XS, Liang HG, Kuang TY (1991) The Comparison of water-splitting activity, DCIP photoreduction activity and some property of photosystem II particles from different plants. *Acta Phytophysiol Sin* 17(4):342–348, (in Chinese)
25. Du LF, Tang XS (1992) Isolation and properties of oxygen-evolving photosystem II reaction center complex. *Acta Phytophysiol Sin* 18(1):17–23, (in Chinese)
26. Hong FS (2004) Effect of  $Eu^{3+}$  on photosystem II spectral characteristics from spinach. *Biol Trace Elem Res* 100:186–196
27. Su MY, Wu X, Liu C, Qu CX, Liu XQ, Chen L, Huang H, Hong FS (2007) Promotion of energy transfer and oxygen evolution in spinach photosystem II by nano-anatase  $TiO_2$ . *Biol Trace Elem Res* 119(2): 183–192
28. Zhao NM, Zhou HM (2000) Biophysics. China Higher Education Press and Springer, Beijing, pp 299–309, (in Chinese)
29. Barber J, Chapman DJ, Telfer A (1987) Characterization of a PS II reaction center isolated from the chloroplasts of *Pisum sativum*. *FEBS Lett* 220:67–73
30. Seibert M, Picorel R, Rubin AB, Connolly JS (1988) Spectral, photophysical, and stability properties of isolated photosystem II reaction center. *Plant Physiol* 87:303–306
31. Guo YJ (1983) In fluorescence technique and its application to molecular biology. Science, Beijing, p 188, (in Chinese)
32. Buchanan BB, Gruissem W, Jones RL (2002) Biochemistry and molecular biology of plants. Science Press, American Society of Plant Physiologists, Beijing, pp 610–616