Thallium Induces Morphological Changes in the Photosynthetic Apparatus of *Synechocystis* sp. PCC6803

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Abstract: The aim of this study was to elucidate the mechanism of thallium (Tl) ion toxicity in photosynthetic organisms. The physiological and biochemical responses to Tl exposure were analyzed in the cyanobacterium *Synechocystis* sp. PCC6803, which is a widely used model to study photosynthesis. We examined the photosynthetic activities of Tl^+-exposed cells, the extent of Tl accumulation, and the properties of membrane lipids. Exposure to Tl^+ at 2.0 and 5.0 for 24 h decreased the net photosynthetic activities of cells to 92% and 34%, respectively. After exposure to 2.5 μM Tl^+, cells concentrated the Tl to 20.8 μM on a packed cell volume basis. Exposure of *Synechocystis* to 0–2.5 μM Tl^+ resulted in an approximately 9-fold concentration factor. Treatment with 2.0 μM Tl^+ for 48 h decreased the total lipid content of the cells by 38%. Further, we observed the ultrastructure of cells treated with Tl^+. The cells exposed to 5 μM Tl^+ for 24 h showed thylakoid membrane fragmentation and generated less-dense particles following osmium staining. During this time, the net photosynthetic oxygen evolution of the cells was reduced to 34%. These results suggest that the accumulation of Tl in cells affects the integrity of the photosynthetic apparatus.

Keywords: Thallium (Tl); Toxicity; Ultrastructure; Cyanobacteria; Thylakoid

Introduction

Thallium (Tl) is a heavy metal belonging to the aluminum group. Tl is anthropogenicaly produced as a byproduct of metal mining/smelting processes or coal combustion and is released into the environment (Lis *et al*., 2003; Xiao *et al*., 2004; Yang *et al*., 2005). In aquatic systems, Tl exists in 2 oxidation states: monovalent (Tl^+), which is the predominant state, and trivalent (Tl^{3+}) (Kaplan and Mattigod, 1998). Tl^+ is highly toxic to plants, animals, and humans (IPCS, 1996). To study the mechanism of Tl^+ toxicity in photosynthetic organisms, we analyzed the physiological and biochemical responses to Tl^+ exposure in cyanobacterium *Synechocystis* sp. PCC6803, a model for photosynthetic organisms. We previously reported that the half maximal inhibitory concentration (IC_{50}) of Tl^+ for growth is approximately 1.0 μM and that 72 h incubation with 2.5 μM Tl^+ decreases the concentration of chlorophyll *a* and phycobiliproteins in each cell by 71% and 94%, respectively (Aoki *et al*., 2008). In this study, we examined Tl accumulation and its effects on the photosynthetic activities and properties of the cell membrane lipids in *Synechocystis* sp. PCC6803.

Materials and Methods

Thallium mononitrate, $TlNO_3$ (Wako Pure Chemical Industries Ltd., Tokyo, Japan) was exposed to the glucose-tolerant wild-type of cyanobacterium *Synechocystis* sp. PCC6803 at various concentrations. The cyanobacterial cells were heterotrophically grown at 30 °C with constant shaking at 120 rpm in BG-11 medium described by Allen (1968); the medium was supplemented with 5 mmol glucose and buffered with 30 mmol HEPES-NaOH, pH 7.5. The cells were illuminated with 30 μmol photons m^{-2} s^{-1} using Biolux fluorescent lamps (BR-A, NEC Corp., Tokyo, Japan).

T. Kuang et al., *Photosynthesis Research for Food, Fuel and the Future*

Cell growth of cells was monitored by measuring the optical density at 730 nm. The carbon dioxide-dependent photosynthetic activities of cells were measured using an Clark-type oxygen electrode (Hansatech Instruments Ltd., Norfolk, UK) under an illumination of 1,000 μmol photons m^{-2} s^{-1}. The Tl concentration of the cells was analyzed by inductively coupled plasma mass spectrometry (ICP-MS) after acid mineralization of the samples in a heating block. Lipids were extracted from the cells using the method described by Bligh and Dyer (1959). Total lipids were separated using normal-phase thin layer chromatography with chloroform ($CHCl_3$): methanol (MeOH): aqueous ammonia (NH_{3aq}) = 13:7:1 (v/v/v) as the solvent system. The separated lipids were methyl esterified with 5% hydrochloric acid (HCl) in MeOH. Subsequently, the fatty acid content of the methyl esters was determined using a gas chromatography/flame ionization detector (GC/FID). Aracidic acid methyl ester was used as the internal concentration standard. The ultrastructure of the cells was observed using a transmission electron microscope (TEM). Cells were fixed by 2% (v/v) glutaraldehyde and 1% (w/v) osmium tetraoxide (OsO_4), dehydrated with acetone series, and embedded in EPON-812 epoxy resin at 60 °C for 24 h. Samples were thin sectioned (60–90 nm) using ultramicrotome equipped with a diamond knife and stained with platinum blue (Nisshin-EM, Tokyo, Japan) and lead acetate. The sections were then examined by electron micrography.

Table 1 Photosynthetic activities of Tl-exposed *Synechocystis* sp. PCC6803 cells.
Following exposure to 0–5 μM Tl for 24 h, the carbon dioxide-dependent photosynthetic activities of the cells were measured using an oxygen electrode under an illumination of 1,000 μmol photons m^{-2} s^{-1}. Gross photosynthetic activity was obtained by adding the oxygen consumption in the dark to the net oxygen evolution. Three independent experimental data were averaged.

Thallium concentration	Net photosynthetic activity		Gross photosynthetic activity	
	Cell basis	Chlorophyll basis	Cell basis	Chlorophyll basis
[μM]	[% of control]	[% of control]	[% of control]	[% of control]
0.0	100	100	100	100
2.0	92	99	92	99
5.0	34	47	40	59

Results and Discussion

The photosynthetic activities of Tl-exposed *Synechocystis* sp. PCC6803 cells are shown in Table 1. Exposure to 2.0 and 5.0 μM Tl^+ for 24 h decreased the net photosynthetic activities of the cells to 92% and 34%, respectively. The inhibitory effects of Tl^+ on the photosynthetic activities per cell were stronger than those on oxygen evolution, as estimated on the basis of the chlorophyll content. Gross photosynthetic activities were also decreased by Tl^+ exposure, but the inhibitory effect was less than that observed on net photosynthetic activities as a result of the increase in respiratory activity in the dark. Tl^+ decreases the content of photosynthetic pigments in each cell (Aoki *et al*., 2008). However, these results suggest that there are other causes for decrease in photosynthetic activities by Tl^+ treatment; these causes cannot be explained by the decrease in photosynthetic pigments. The results also suggest that the respiratory activity of cells is not inhibited by the Tl^+ exposure conditions used.

To clarify the relation between Tl^+ treatment conditions and Tl accumulation levels in cells, Tl concentration of cells was measured. Tl uptake of cells depended on Tl concentration that the cells were exposed to. Approximately 9-fold concentration factors were constantly observed in the 0.1–2.5 μM of Tl^+ exposure conditions, suggesting that Tl is actively transported into cells. The Tl ion has a similar ionic radiuses that of the potassium ion (Tl^+, 1.49 Å; K^+, 1.33 Å). It has been shown that Tl^+ can be transported by membrane Na^+, K^+-ATPase (Landowne, 1975; Skulskii *et al*., 1978; McCall *et al*., 1985). Therefore, the mechanism of Tl uptake in *Synechocystis* could be via an active ion transport system such as membrane Na^+, K^+-ATPase.

Furthermore, to investigate the effect of Tl^+ uptake into the cell on the photosynthetic membrane, we analyzed the membrane lipid content of the cells.

Exposure to Tl decreases the membrane lipid content of the cells. Treatment with 2.0 μM Tl^+ for 48 h decreased the total lipid content of the cells by 38%. The individual polar lipid contents, determined on the basis of cell volume, decreased remarkably on exposure to 1.0–2.0 μM of Tl^+. Marked reductions were observed, especially in monogalactosyl-diacylglycerol (MGDG) and sulfoquinovosyl-diacylglycerol (SQDG) by 49% and 33%, respectively. On the other hand, no significant changes were observed in the total fatty acid composition of the cells exposed to Tl^+. MGDG is an integral constituent of different photosynthetic pigment-protein complexes (Dörmann and Hörzl, 2009), and SQDG is essential for photoautotrophic growth of *Synechocystis* (Aoki *et al*., 2004). These dynamic changes in the membrane polar lipid composition along with decreased in the photosynthetic activities due to Tl^+ exposure suggest that Tl^+ impairs the integrity of the photosynthetic apparatus of the cell.

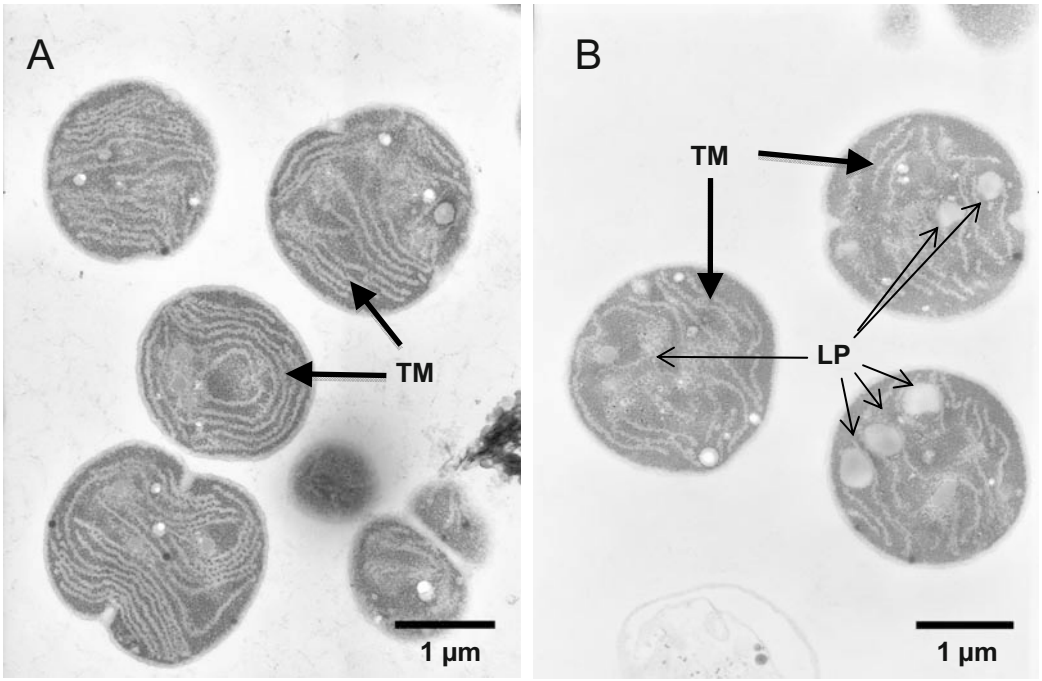

Fig. 1 Effect of Tl^+ on the ultrastructure of *Synechocystis* sp. PCC6803 cells.
The cells grown for 24 h without (A) or with 5 μM Tl^+ (B) were fixed by glutaraldehyde and osmium tetraoxide. The fixed cells were dehydrated in acetone series and embedded in epoxy resin. Samples were sectioned and observed under a transmission electron microscope. TM, thylakoid membrane; LP, low electron-dense particle.

We then observed the ultrastructure of the cells treated with Tl^+ using TEM. The control cells not exposed to Tl had cell diameters of approximately 2 μm and semi-concentrically stacked thylakoid membranes (Fig. 1A). In contrast, exposure to 5 μM Tl for 24 h induced unusual changes in the cell ultrastructure but not in the cell diameter (Fig. 1B). Exposure to Tl caused fragmentation and virtual disappearance of the thylakoid stacks in the *Synechocystis* cells (shown as TM in Fig. 1). Furthermore, several particles of approximately 100–300 nm diameters with low electron density were observed in the cells exposed to Tl^+ (shown as LP in Fig. 1B). In yeast and plant cells showing heavy metal tolerance, the vacuole is generally considered the main storage site for metals (summarized in Martinoia *et al*., 2007). Although the role of the particles produced because of Tl^+ treatment in *Synechocystis* is still unclear, these particles may play a role in easing the Tl^+ stress response. While elucidating the mechanism of Tl toxicity, the interaction between Tl accumulation and the particles in response to Tl^+ should also be considered.

Based on the experimental findings, the following mechanism is proposed for Tl toxicity in *Synechocystis* sp. PCC6803 cells, Tl from the medium is imported into the cell; Tl decreases the membrane lipid content of the cell. This decrease causes abnormalities in the cell ultrastructure, thereby leading to a decrease in the concentration of photosynthetic

pigments and photosynthetic activity. Thus, Tl inhibits cell growth.

Conclusion

Understanding the mechanism of Tl^+ toxicity in photosynthesis in the model *Synechocystis* sp. PCC6803 is important for advancing the science of heavy metal stress responses. In this study, *Synechocystis* cells exposed to Tl^+ showed thylakoid membrane fragmentation and generated less electron-dense particles, resulting in reduced net photosynthetic oxygen evolution of the cells. These results suggest that the accumulation of Tl in the cell affects the integrity of the photosynthetic apparatus.

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