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ORIGINAL ARTICLE

External spermine prevents UVA-induced damage of *Synechocystis* sp. PCC 6803 via increased catalase activity and decreased H_2O_2 and malonaldehyde levels

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Abstract

Common polyamines, putrescine (Put), spermidine (Spd), and spermine (Spm), are cationic compounds known as beneficial factors for many cellular processes including cell division, proliferation, differentiation, and stress response in all living organisms. Effects of exogenous Spm on the protective responses of *Synechocystis* sp. PCC 6803 exposed to UVA were investigated. The presence of 0.5 mM Spm in the culture medium significantly reduced cell growth after 60 min under white light condition but protected the cells after growing for 60 min under UVA. The stress-tolerant response of *Synechocystis* cells represented by the ratio of putrescine/spermidine (Put/Spd) showed about a 6-fold increase after 60 min UVA in the presence of Spm. In addition, those levels of chlorophyll *a*, carotenoids, and photosynthetic oxygen evolution were increased by Spm supplementation in UVA-treated cells. Exogenous Spm induced the activity of catalase but not superoxide dismutase in cells under UVA treatment. On the other hand, Spm treatment enabled cells to apparently decrease the intracellular free radical H_2O_2 and malonaldehyde (MDA) levels. Overall results suggested that Spm supplementation could protect *Synechocystis* sp. PCC 6803 cells via the increase of Put/Spd ratio and the reduction of both H_2O_2 and MDA levels in conjunction with the induction of catalase activity. Interestingly, UVA-treated cells as compared to non-treated cells with exogenous Spm showed a decrease of Spm with an increase of Put and no change in Spd. This suggested the back conversion of Spm to Spd and finally to Put as cellular mechanism in response to UVA.

Keywords Exogenous spermine · UVA irradiation · *Synechocystis* sp. PCC 6803 · Antioxidant enzyme

Introduction

Cyanobacteria utilize sunlight to support their growth via photosynthesis, and consequently, they are exposed to ultraviolet (UV) radiation present in the sunlight. The UV region, which encompasses 100–400-nm wavelength range, is classified mainly into three bands: UVA (315–400 nm), UVB (280–315 nm), and UVC (100–280 nm). UV radiation reaching the Earth surface is mostly composed of UVA with a small composition of UVB, as long as certain ozone amount exists in atmosphere layer to present the penetration of UVC (Stapleton 1992). Living organisms are mainly affected by more than 95% of UVA in sunlight. UVA radiation can generate reactive oxygen species (ROS) (Cadet et al. 2009), leading to the oxidative stress especially when the imbalance between of intracellular ROS levels and the ROS detoxification system occurs. Negative effect of UVA radiation upon various organisms has been reported, such as a decreased growth of *Synechocystis* sp. PCC 6803 (Jantaro et al. 2011), a decreased chlorophyll fluorescence in marine diatoms *Phaeodactylum tricornutum* and *Chaetoceros muelleri* (Liang et al. 2006), and an inhibition of photosystem II photochemistry of wheat seedlings (Joshi et al. 1997). On the other hand, the beneficial effects of UVA were also reported, such as the accumulation of unsaturated fatty acids in *Nitzschia closterium* and *Isochrysis zhangjiangensis* (Huang and Cheung 2011) and in *Phaeodactylum tricornutum* and *Chaetoceros muelleri* (Liang et al. 2006). Since UVA radiation has been known to generate oxidative stress, there are several mechanisms to cope with this stress such as the

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increased activity of antioxidant enzymes including catalase in *Pseudomonas aeruginosa* (Pezzoni et al. 2014) and the biosynthetic induction of antioxidant compounds such as carotenoids (Huang and Cheung 2011), ascorbate (White and Jahnke 2002), and polyamines (Ha et al. 1998).

Polyamines are cationic compounds at physiological pH present in all living organisms. They are key factors for many cellular processes including cell division by stabilizing DNA structure, cell proliferation, and differentiation, as well as stress response. They function as free radical scavengers and antioxidants against the environmental stress-derived oxidative damage (Groppa and Benavides 2008; Schweikert and Burritt 2015). Usually, putrescine (Put), spermidine (Spd), and spermine (Spm) are common polyamines inside the cells. Polyamines are also involved in various cellular regulatory processes such as cell differentiation and cell proliferation (Evans and Malmberg 1989). The supplementation of different kinds of polyamines to stressed organisms significantly affects cellular metabolism. For instance, in higher plants such as tobacco (*Nicotiana tabacum* cv. Xanthi), putrescine was shown to be the ATP synthesis stimulator whereas Spd and Spm were stimulators of non-photochemical quenching (Ioannidis and Kotzabasis 2007). Regarding to spermine with beneficial effects, Spm addition has been studied mostly in mammals with and without oxidative stress (Cao et al. 2015; Liu et al. 2014; Wu et al. 2017). In cyanobacteria, Spd addition was shown to enhance the growth of *Synechocystis* cells exposed to UVA, UVB, and UVC irradiations (Jantaro et al. 2014). It is therefore of interest to find out whether the addition of Spm, which is present in small amounts intracellularly, would affect *Synechocystis* cells under UV stress. In the present study, we showed that the UVA could decrease the growth and photosynthetic efficiency of *Synechocystis* sp. PCC 6803 cells. However, the negative effect of UVA on cell growth could be alleviated by Spm supplementation with the consequent reduction of hydrogen peroxide and malonaldehyde levels inside *Synechocystis* cells, as well as the increase of catalase antioxidant enzyme activity.

Materials and methods

Cell culture and UVA radiation treatment

The cultivation of *Synechocystis* sp. PCC 6803 was carried out in BG_{11} liquid medium until reaching mid-log phase under normal growth condition. The optimum growth condition was set at 28–30 °C with 160 rpm on a rotary shaker, under continuous white light illumination of 40–50 μmol m^{-2} s^{-1} as normal growth light. Cell growth was determined by measuring optical density at 730 nm. The Spm supplementation was obtained at a final concentration of 0.5 mM in BG_{11} medium (Raksajit et al. 2009). The UVA treatment was performed by irradiation over a flat bottom chamber containing mid-log phase cells ($OD_{730\ nm}$ of 0.5) in Spm-supplementing BG_{11} medium. The UVA single lamp at 365 nm was employed with the intensity of 11.0–13.0 W m^{-2}.

Determinations of chlorophyll *a* and carotenoids

Cell pellets from 1 ml of *Synechocystis* culture under normal growth light or under UVA were collected after centrifugation at 2790×*g* for 10 min and subsequently extracted using dimethylformamide (DMF). The pigment measurement of DMF-extracted sample was detected spectrometrically for carotenoids and chlorophyll *a* according to Chamovitz et al. (1993) and Moran (1982), respectively.

Polyamine extraction and detection

The cells harvested from 50 ml culture at mid-log growth were extracted for total polyamines by 5% perchloric acid (PCA) on ice bath. Two fractions containing "PCA-soluble part" and "PCA-insoluble part," representing total intracellular polyamines, were separated and further derivatized by benzoylation method (Jantaro et al. 2003). Those derivatized polyamine fractions were detected using high-performance liquid chromatography (HPLC). The internal standard, 1,6-hexane-diamine, was added into the reaction and extracted simultaneously with extracted sample (modified from Flores and Galston 1982). The HPLC detection was performed using a C-18 reverse phase column and a UV-Vis detector at 254 nm. The mobile phase was a gradient of 50–80% methanol in water with a flow rate of 0.5 ml per min.

Total protein extraction and enzyme activity assays

Synechocystis cell culture (100 ml) was harvested and further extracted as crude protein. The cell pellets from harvested cells were washed once by 10 mM Hepes-NaOH (pH 7.5) containing 5 mM NaCl and 2 mM NaEDTA after centrifuging with a speed of 2790×*g* for 10 min at 4 °C. Next, those washed pellets were resuspended in a reaction mixture of 10 mM Hepes-NaOH (pH 7.5) buffer containing 20% (*w*/*v*) sucrose and 0.2% (*w*/*v*) lysozyme at the ratio of 1:2 (cells/buffer, *w*/*v*). The lysozyme digestion was performed optimally at 37 °C for 30-min incubation time. Later, pre-cooled glass beads were then added into the reaction tube and vortexed vigorously at least five times by interval keeping on ice. After crude protein extraction was completed, the supernatant fraction was obtained after centrifugation at 2790×*g* for 10 min at 4 °C. The protein concentration was measured according to Bradford (1976). This total protein extract was further used for the assay of enzyme activity.

Catalase (CAT) activity was determined by measuring H_2O_2 consumption in the reaction (modified from Dhindsa

et al. 1981). The reaction mixture comprised 50 μg of extracted protein dissolved in 50 mM potassium phosphate buffer (pH 7.0). After the addition of 0.15 mM H_2O_2, the H_2O_2 consumption was determined by measuring the absorbance at 240 nm for 5 min. Specific activity was expressed as micromoles of decomposed H_2O_2 per minute per milligram of protein.

SOD activity was determined by measuring the inhibition in photochemical reduction of nitroblue tetrazolium (NBT) in the reaction (modified from Beauchamp and Fridovich 1971). The extracted protein (50 μg) was added into a reaction mixture containing 50 mM phosphate buffer (pH 7.8), 50 mM sodium carbonate, 13.3 mM L-methionine, 0.1 mM EDTA, 75 mM NBT, and 2 μM riboflavin. The reaction was started by exposing to light for 15 min and subsequently stopped by incubation under darkness for 10 min before measuring the absorbance at 560 nm. One unit of SOD inhibits the rate of increase in A_{560} by 50% under the assay conditions. The specific activity was expressed in units per min per mg protein.

Hydrogen peroxide determination

H_2O_2 contents were measured according to Jana and Choudhuri (1982). *Synechocystis* cell culture (100 ml) was harvested and centrifuged at 2790×*g* for 10 min. The cell pellet was resuspended in sodium phosphate buffer (50 mM, pH 6.5) and further vortexed with pre-cooled glass beads. The supernatant obtained after centrifugation at 2790×*g* for 10 min was then mixed with 0.1% (*w*/*v*) titanium chloride in 20% (*v*/*v*) H_2SO_4. The intensity of yellow color was developed for 1 min before measuring A_{410} spectrometrically.

Lipid peroxidation determination

The lipid peroxidation was determined by monitoring the generation of malondialdehyde (MDA) content using thiobarbituric acid (TBA) reaction (modified from Heath and Packer 1968). The reaction mixture consisting of 50 μg extracted protein and 0.5% (*w*/*v*) TBA in 20% (*w*/*v*) trichloroacetic acid was boiled at 95 °C for 30 min and then quickly cooled down on ice bath. After quick centrifugation, the solution was taken and measured at A_{532} and A_{600}. MDA level was normalized from the absorbance difference to total protein content.

Statistics

Three replicates of independent experiments were performed in this study. The data are expressed as means ± standard deviation. Statistical significances between treatments were evaluated using Student's *t* test ($p < 0.05$).

Results

Effect of exogenous Spm on cell growth, intracellular pigments, and oxygen evolution

The decrease of *Synechocystis* cell growth was observed under UVA exposure for 60 min compared to the control under normal growth light (Fig. 1a). Cell growth was restored upon addition of 0.5 mM Spm to the growth medium. It is noted that 0.5 mM Spm supplementation also damaged cells under normal white light condition. The level of intracellular Spm increased drastically after 5 min of exogenous Spm treatment giving the highest Spm content followed by the decrease until 60 min for about 30–40% remaining compared to that highest level (Fig. 2). UVA radiation had no effect on the level of intracellular Spm which was detected in traces, in concentrations not significantly different from the basal levels. Cells under white light and UVA radiation grown for 60 min showed no changes of intracellular Put/Spd ratio (Fig. 2, insets). In the presence of Spm, this Put/Spd ratio was increased about 3-fold at 60 min under normal white light condition. Interestingly, a much higher increase of Put/Spd ratio about 5-fold at 60 min was observed in the presence of Spm under UVA radiation.

UVA radiation was decreased both chlorophyll *a* and carotenoid contents compared to the control (Fig. 1b, c). In contrast, exogenous Spm slightly increased these two intracellular pigments under UVA radiation. It is noted that exogenous Spm decreased both chlorophyll *a* and carotenoid contents under normal growth light. Under UVA radiation, cells showed an increase of photosynthetic O_2 evolution in the presence of Spm (Fig. 1d). On the other hand, under normal growth light, the photosynthetic O_2 evolution was increased in the absence of exogenous Spm.

Effect of exogenous Spm on H_2O_2 and MDA levels in *Synechocystis* cells under UVA radiation

The increased level of intracellular H_2O_2 content was slightly induced by UVA compared to the control (Fig. 3a). Interestingly, exogenous Spm could significantly reduce intracellular H_2O_2 under both UVA and normal growth light conditions. On the other hand, the level of malonaldehyde (MDA), the final product of lipid peroxidation, was significantly increased by UVA radiation (Fig. 3b). The Spm-added cells showed a significant reduction of MDA level under UVA exposure, whereas a slight decline of MDA level was observed under normal growth light condition.

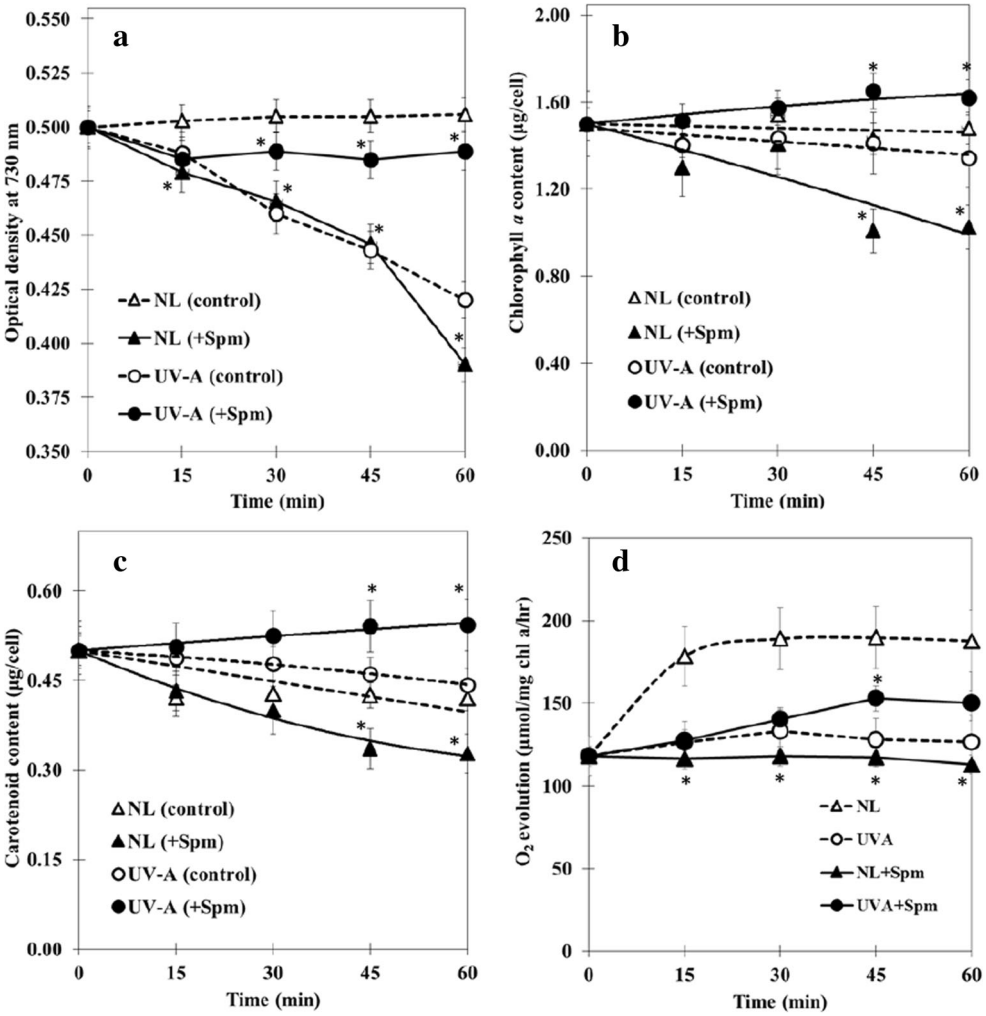

Fig. 1 UVA radiation effect on *Synechocystis* cell growth (**a**), intracellular pigment contents including chlorophyll *a* (**b**) and carotenoids (**c**), as well as oxygen evolution rate (**d**) of *Synechocystis* cells grown in BG_{11} medium with or without Spm supplementation. Data represent mean ± S.D. ($n = 3$). The statistical difference of the results between with and without Spm supplementation is indicated by an asterisk at $^*P < 0.05$

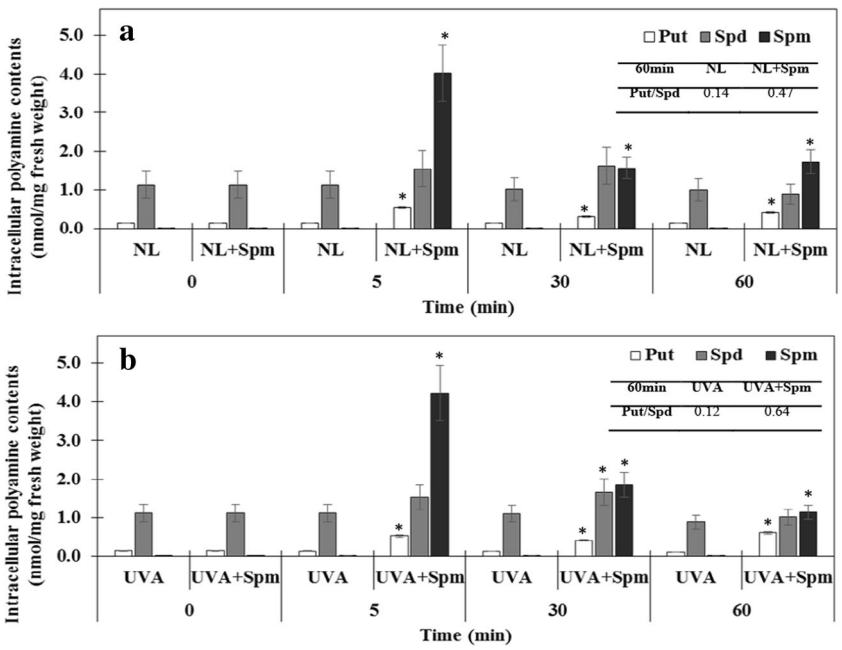

Fig. 2 Intracellular polyamines of *Synechocystis* sp. PCC 6803 grown in BG_{11} medium with or without Spm supplementation under normal growth light (NL) and UVA radiation. Data represent mean ± S.D. ($n = 3$). The statistical difference of the results between with and without Spm supplementation is indicated by an asterisk at $^*P < 0.05$. Insets in **a** and **b** show the ratio of Put/Spd content at 60-min treatment

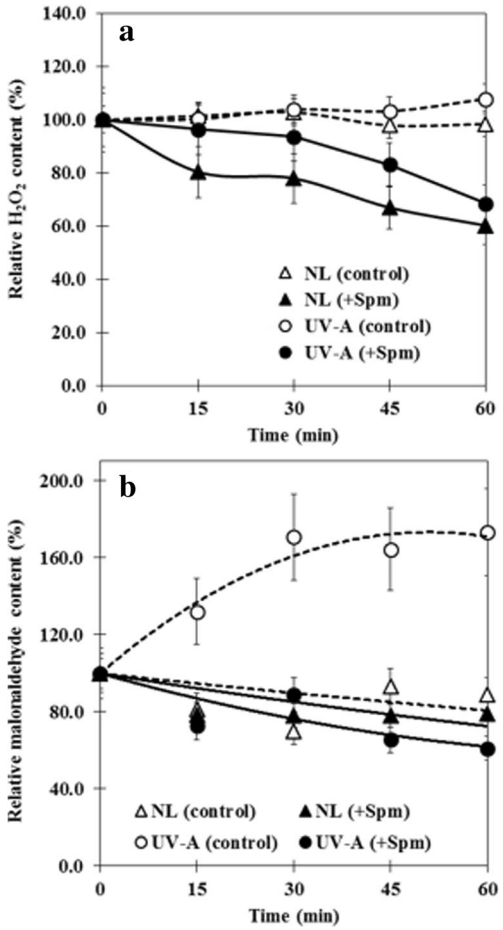

Fig. 3 The relative contents of hydrogen peroxide (**a**) and malonaldehyde (**b**) generated by UVA radiation in *Synechocystis* cells grown in BG_{11} medium with or without Spm supplementation. The BG_{11} media with Spm at 0.5 mM concentration (closed symbols) and without Spm (open symbols) were used to grow *Synechocystis* sp. PCC 6803 cells under normal growth light (NL, triangle) and UVA radiation (circle). The 100% of each control data is derived from the hydrogen peroxide and malonaldehyde of 70 nmol per mg protein and 0.11 nmol per mg protein, respectively. Data represent mean ± S.D. ($n = 3$)

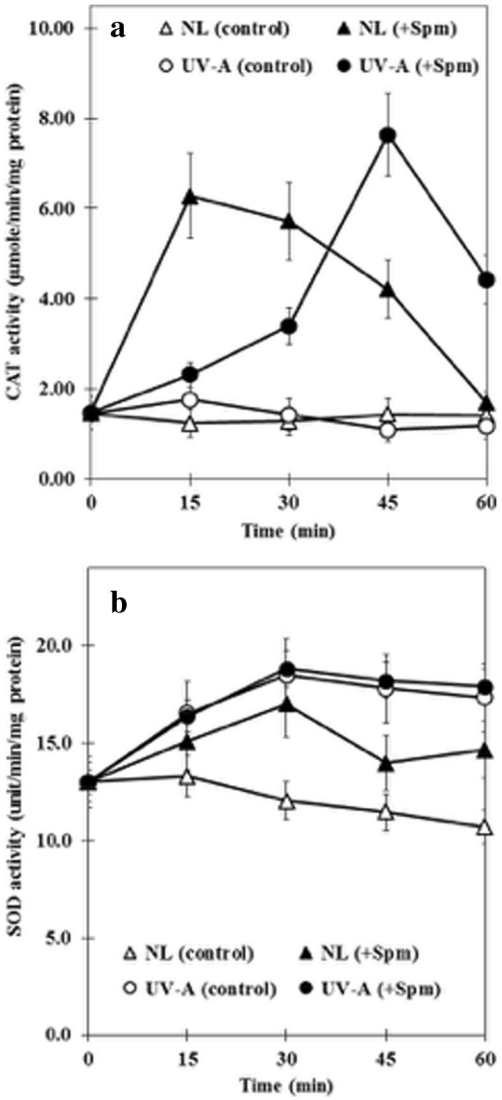

Fig. 4 Effect of UVA radiation on catalase (CAT) and superoxide dismutase (SOD) activities in *Synechocystis* cells grown in BG_{11} medium with or without Spm supplementation. The BG_{11} media with Spm at 0.5 mM concentration (closed symbols) and without Spm (open symbols) were used to grow *Synechocystis* sp. PCC 6803 cells under normal growth light (NL, triangle) and UVA radiation (circle). Data represent mean ± S.D. ($n = 3$)

Effect of exogenous Spm on the activities of CAT and SOD in *Synechocystis* cells under UVA radiation

The CAT activity was similar in cells under normal growth light and under UVA radiation (Fig. 4a). However, Spm-supplemented cells showed a significant increase of CAT activity under both normal growth light and UVA exposure with a decline at 60 min. It is noted that under UVA, the induction of CAT activity by Spm occurred later than that under normal growth light. On the other hand, UVA radiation strongly increased SOD activity, whereas no further increase in the activity was observed upon Spm supplementation (Fig. 4b). In contrast, the presence of Spm significantly increased SOD activity under normal growth light.

Discussion

Our previous report revealed the capability of polyamine Spd addition on the alleviation of UV-induced growth inhibition in *Synechocystis* cells via the reduction of hydrogen peroxide and malonaldehyde levels (Jantaro et al. 2014). Exogenous Spd is the dominant polyamine inside the cells and is less toxic to *Synechocystis* cells when compared to Spm. Recently, the Spm application in pea plants was suggested to protect plant from UVC damage by maintaining normal growth and stabilization of cell membranes, as well as non-enzyme antioxidant stimulation (Todorova et al. 2013). We demonstrated that the

intracellular accumulation of Spm was increased abruptly after 5 min Spm supplementation plus UVA, whereas after 60 min treatment, the level of intracellular Spm decreased to about 30–40% compared to the highest content (Fig. 2b). The decrease of intracellular Spm is due to its conversion to Spd and finally to Put, resulting in the increased ratio of Put/Spd which contributed to the maintenance of cell survival under UVA plus exogenous Spm condition as shown in Fig. 1a. Previously, supplementation of Spd plus UVA also resulted in the increased ratio of Put/Spd (Jantaro et al. 2014). It is worth pointing out that UVA plus exogenous Spm has higher Put level than that by NL plus exogenous Spm with unchanged level of Spd (Fig. 2). This suggested that UVA induced the conversion of Spm to Spd and finally to Put. Since Spd level was unchanged, it is likely that the increased level of Put plays a role in the upregulation of signaling-related genes (Pál et al. 2015). Recently, it has been reported that the polyamine back-conversion pathway was catalyzed by polyamine oxidase in *Synechocystis* sp. PCC 6803 (Samasil et al. 2017). Not only the recovery of cell growth but also the increase of photosynthetic efficiency represented by higher O_2 evolution (Fig. 1d) were observed in UVA treated cells in the presence of external Spm. We also found that exogenous Spm efficiently increased the accumulation of pigments such as chlorophyll *a* and carotenoids during UVA stress. Thus, the improved survival of *Synechocystis* was partly attributed to higher pigment accumulation induced by exogenous Spm under UVA stress. In line with the present study, polyamines were found to be associated with thylakoid membrane which functionally played a protective role on the photosynthetic apparatus in higher plant (Lütz et al. 2005). Furthermore, Spm effectively stimulated higher chlorophyll content, electron transport, and energy transfer between PSII and PSI than did Spd and Put (Ioannidis and Kotzabasis 2007). It should be noted that after 60-min UVA treatment, there was no change in polyamine content (Fig. 1a, NL and Fig. 1b, UVA). This suggested that polyamine is not directly responsible for UVA stress tolerance in *Synechocystis* cells. However, upon Spm addition with UVA treatment, cell survival was maintained (Fig. 1a) as a consequence of the increase of carotenoids content (Fig. 1c). Carotenoids can act as UV-absorbing molecules, thus reducing the detrimental effect on the cell. Other UV-screening compounds are mycosporine-like amino acids (MAAs) and scytonemin whose biosynthesis and accumulation have been reported in several cyanobacteria except *Synechocystis* (Rastogi et al. 2015; Singh et al. 2010).

The generation of hydrogen peroxide (H_2O_2) which is a reactive oxygen species (ROS) was induced slightly by UVA radiation (Fig. 3a). It was also clear that Spm decreased H_2O_2 accumulation not only under UVA radiation but also under normal growth light condition. Spm seemed to exert its effect on ROS reduction. Spm addition could inhibit the Cu(II)/H_2O_2-dependent oxygen-radical generating system, thus supporting the role of Spm as an antioxidant or a radical scavenger (Ha et al. 1998). On the other hand, malonaldehyde (MDA) which represents the end product of lipid peroxidation is an indicator of the damage on cell membrane containing mainly lipids. UVA radiation induced the increase of MDA content compared to that under normal growth light (Fig. 3b). Our results are in agreement with those of Unal et al. (2008). Spm addition to UVA-exposed cells significantly decreased their MDA content (Fig. 3b). The decrease of MDA level under exogenous Spm supplementation could be due to the binding of imported Spm to thylakoid membrane leading to the reduction in lipid peroxidation, as has been reported in higher plant (Bestfort et al. 1993). Moreover, polyamines especially Spd and Spm had been shown to reduce apparently the MDA levels in osmotically stressed oat-leaf tissue (Borrell et al. 1997). Moreover, Spm administration to rat enhanced the antioxidant capacity and reduced MDA content in the liver and spleen, as well as increased their catalase activity under oxidative stress (Wu et al. 2017).

Another beneficial aspect of Spm supplementation under UVA is the increase of CAT activity (Fig. 4a). This result was different from that of Spd supplementation in previous study where no increase of CAT activity was observed (Jantaro et al. 2014). UVA radiation itself could not induce the CAT activity as compared to that under non UVA condition. The response of *Synechocystis* cells to environmental stress with regard to CAT activity varied depending on their detoxifying mechanisms. For example, the microcystin toxin from cyanobacteria *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* could induce CAT activity of *Synechocystis* sp. PCC 6803 (Vassilakaki and Pflugmacher 2008). In the bacterium *Pseudomonas aeruginosa*, CAT activity was the main antioxidant enzyme responsible for cell survival under UVA radiation (at 20 W m^{-2} for 180 min) compared to that of catalase-deficient strain (Pezzoni et al. 2014). For the activity of another antioxidant enzyme, superoxide dismutase (SOD), its increase was highly induced by UVA radiation alone compared to control condition (Fig. 4b). Similar observation was also recently reported for a N_2-fixing cyanobacterium *Anabaena siamensis* TISTR 8012 exposed to UV radiation showing the increase of the antioxidant enzyme activities including SOD, CAT, and peroxidase (Rastogi et al. 2014). Interestingly, Spm supplementation apparently enhanced SOD activity of *Synechocystis* cells exposed to normal growth light condition, whereas no changes in SOD activity were observed under UVA radiation.

Altogether, there are at least four possible mechanisms by which Spm could reduce UVA-induced oxidative damage in this study. Spm might interfere with the lipid peroxidation process which generate MDA molecule as a final product. Spm may possibly act as a free radical scavenger, at least with H_2O_2. Spm can act as an inducer to those antioxidant enzymes including CAT and SOD, although no effect on SOD was

observed under UVA radiation. Lastly, Spm can be a signaling molecule to increase the biosynthesis of carotenoids which can act as both UV-absorbing and antioxidant molecules. Another possible cellular mechanism in response to UVA radiation with exogenous Spm would be due to the UVA-induced back conversion of Spm to Spd and finally to Put as evident by the increase of Put/Spd ratio after UVA treatment. This is also substantiated by the observation that UVA alone did not increase Put (Fig. 2a, b). However, in order to fully understand the in vivo mechanism of action of Spm against UV stress, particularly the mechanism regarding the combined effect of UV and exogenous Spm, further study employing molecular genetics such as polyamine oxidase knockout is needed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent is not required in this study.

Research involving human participants and/or animals This article does not contain any studies with human participants or animals performed by any of the authors.

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