Pak. J. Bot., 48(6): 2587-2593, 2016.

UV-B RADIATION INHIBITS THE PHOTOSYNTHETIC ELECTRON TRANSPORT CHAIN IN *CHLAMYDOMONAS REINHARDTII*

WENKAI CAI[1,3], XIANG GAO[1,3], JINLU HU[1,3], LANZHOU CHEN[2], XIAOYAN LI[1], YONGDING LIU[1] AND GAOHONG WANG[1*]

[1]*Key Laboratory of Algal Biology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, CHINA;*
[2]*School of Resource and Environmental Science, Wuhan University, Wuhan, 430079, CHINA;*
[3]*University of Chinese Academy of Sciences, Beijing 100039, CHINA*
[*]*Corresponding author's email: space@ihb.ac.cn; Tel/Fax: +86-27-6878-0036*

Abstract

UV radiation of sunlight is one of harmful factors for earth organisms, especially for photoautotrophs because they require light for energy and biomass production. A number of works have already been done regarding the effects of UV-B radiation at biochemical and molecular level, which showed that UV-B radiation could inhibit photosynthesis activity and reduce photosynthetic electron transport. However quite limited information can accurately make out inhibition site of UV-B radiation on photosynthetic electron transport. In this study, this issue was investigated through measuring oxygen evolution activity, chlorophyll a fluorescence and gene expression in a model unicellular green alga *Chlamydomonas reinhardtii*. Our results indicated that UV-B radiation could evidently decrease photosynthesis activity and inhibit electron transport by blocking electron transfer process from the first plastoquinone electron acceptors Q_A to second plastoquinone electron acceptors Q_B, but not impair electron transfer from the water oxidizing complex to Q_A. The psbA gene expression was also altered by UV-B radiation, where up-regulation occurred at 2, 4 and 6h after exposure and down-regulation happened at 12 and 24 h after exposure. These results suggested that UV-B could affects D1 protein normal turnover, so there was not enough D1 for binding with Q_B, which may affect photosynthetic electron transport and photosynthesis activity.

Key words: UV-B radiation; Photosynthesis; *Chlamydomonas reinhardtii*; Chlorophyll a fluorescence; psbA gene expression.

Introduction

The level of UV radiation reaching the Earth's surface have significantly increased due to destruction of stratospheric ozone, and in the Polar Regions (ozone holes) this increase is about 6% maximum per decade (Frederick&Lubin, 1988; Madronich *et al*., 1995). UV radiation, especially ultraviolet-B (UV-B, 280–320 nm) in solar light, can affect a multitude of photosynthetic process, including photosynthetic oxygen evolution, photosynthetic pigment synthesis, CO_2 fixation and electron flow transport in the PSII (Sicora *et al*., 2003; Wang *et al*., 2010a; Rastogi *et al*., 2014; Mohajer *et al*., 2015; Sompornpailin & Kanthang, 2015). Some researchers reported that the primary effect of UV-damage occurs at the catalytic Mn cluster of photosystem (Sicora *et al*., 2006), and Mn cluster could be inactivated by released from water-oxidation complex and loss of the oxygen evolving activity under UV-B radiation. But Vass and colleague demonstrated UV-A radiation dominantly damage located at Q_B quinone electron acceptor, leading to inhibition of electron transport (Turcsanyi & Vass, 2000), and they also pointed out that UV-B radiation impairs PSII by modification of Q_B binding site and affects the binding of plastoquinone, which lead to loss its function and the inhibition in the electron transport chain (Vass *et al*., 2005). However, it is unclear about the primary inhibition site of photosynthetic electron transport chain by UV-B radiation, which is expected to be an important component to interpret UV-B damage. In this study, we used *Chlamydomonas reinhardtii* to carry out our research since this alga is a suitable model organism for photosynthesis research (Harris 2001; Merchant *et al*., 2007; Hu *et al*., 2014). We want to address this issue through measuring the biochemical and physiological parameters of *Chlamydomonas reinhardtii* under UV-B radiation, which included: (1) to investigate photosynthesis activity of *Chlamydomonas reinhardtii* under UV radiation; (2) to determine chlorophyll fluorescence of the alga after exposure to UV-B radiation to identify inhibition sites of the photosynthesis system; (3) to study gene expression of photosynthetic reactive center protein and then to discuss inhibition site of UV-B radiation on photosynthetic system in algae.

Materials and Methods

Algal culture and treatment with UV-B radiation: The *Chlamydomonas reinhardtii* wild type (WT) strain CC-125 (kindly donated by Dr. Kenichi Wakabayashi, Tokyo University, Japan) was grown in liquid Tris-acetate-phosphate (TAP) medium (Hema *et al*., 2007), at 23±1°C under aerobic incubation, and continuous illumination using cool fluorescent lights and light intensity of 100 ± 5μmol photo $m^{-2}s^{-1}$.

The artificial UV-B radiation was obtained by a combination of fluorescent lamps (He & Häder, 2002b). UV-B from the power of a 15 watts Cole Parmer UV light (wavelength 312 nm) and cellulose acetate was used to filter out UV-C. The irradiance was 60 μmol photo $m^{-2}s^{-1}$ PAR and UV-B radiation was measured with an EW-97503-00 luminance meter and an EW-97503-52 probe (Cole-Parmer Instruments). The four UV-B radiation treatment levels were 0 (control), 0.2, 0.4 and 0.8 W/m^2.

Determination of photosynthetic oxygen evolution activity: The photosynthetic oxygen evolution rates of *Chlamydomonas reinhardtii* were measured with a Clark-type oxygen electrode (Hansatech Instruments Ltd, UK) according to the manufacturer's instructions. Algae samples were kept at 23°C by a refrigerated circulator and at 100μmol photo $m^{-2}s^{-1}$ illumination under continuous stirring.

Determination of photosynthetic activity by Phyto-PAM: The potential maximum PSII quantum yield (Fv/Fm) was determined by using a Phyto-PAM (Walz, Germany). All samples are needed to be dark-adapted for 15 min at 23°C prior to measurements. Phyto-PAM could measure the minimal fluorescence yield (Fo), the maximal fluorescence yield (Fm) and Rapid light curves (RLC) according to the manufacturer's instructions. From these data, the potential maximum PSII quantum yield (Fv/Fm) were calculated as Fv/Fm = (Fm-Fo)/Fm (Ralph & Gademann, 2005).

Determination of chlorophyll fluorescence by Handy PEA: Chlorophyll a fluorescence was determined at room temperature by a portable Handy-PEA (Instruments Ltd, UK). According to the method described by Strasser and Srivastava (Strasser & Srivastava, 1995), the measurements were carried out at 23°C after a dark adaptation period of 15 min.

Determination of gene expression by quantitative RT-PCR: Total RNA was extracted from algal cells using TRIZOL reagent (Invitrogen, USA) according to the manufacturer's instructions. Then total RNA was extracted by adding RQ1 RNase-Free Dnase (Promega) at 37°C for 30 min to digest DNA. First-Strand cDNA Synthesis were performed using an RT reagent Kit (TaKaRa, Tokyo, Japan). The PCR thermal cycles used were as follows: 5 min at 95°C for cDNA denaturation, followed by 30 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C, and a final elongation was performed at 72°C for 10 min. The 18S rRNA gene was used as the housekeeping gene to normalize the expression levels.

Gene specific PCR primers synthesized by TianyiHuiyuan Life Science and Technology Company (Beijing, China) were used in this study as outlined in Table 1. The primers of 18s rRNA and psbA were used as suggested by Yoshida *et al*. (Yoshida *et al*., 2004) and Teramoto *et al*. (Teramoto *et al*., 2002), respectively.

Table 1. Gene specific PCR primers.

Gene	Primer	Sequence (5′–3′)
18S rRNA	Forward	ACTGCTCTGCTCCACCTTCC
	Reverse	TATTCAGAGCGTAGGCCTGC
psbA	Forward	GGCCAAGGTTCATTCTCTGA
	Reverse	CACCGAATACACCAGCAACAC

Data analysis: All the experiments were carried out for three times with more than three replicates. All data are presented as mean ± SD and significant differences between the control and treated samples were determined by one way ANOVA and multiple comparisons with LSD using SPSS 16.0 for Windows. Differences in gene expression levels were analyzed by △△CT method (Livak & Schmittgen, 2001; Hou *et al*., 2015). In addition, the graphs were obtained using Origin 8.0 data analysis software (Origin Lab Corporation, USA).

Result

UV-B radiation inhibited photosynthesis activity of algae: In order to determine the effects of UV-B exposure on *Chlamydomonas reinhardtii* cells, samples were irradiated with different intensities of UV-B (0.2, 0.4, and 0.8 W/m^2). Figs. 1 and 2 showed respectively the inhibition effect of UV-B radiation on the oxygen evolution rate and maximum quantum yield (Fv/Fm) in alga. Oxygen evolution rate and Fv/Fm are frequently considered as the effective indicator to determinate photosynthesis efficiency in vivo (Longstaff *et al*., 2002). The values of oxygen evolution rate and Fv/Fm in the control group exhibited a relatively stability during whole experiment time, which were different significantly from data in the UV-B radiation group (Figs. 1 and 2). At 0.2 W/m^2 level, oxygen evolution rate values were almost unchanged compared to the control (Fig. 1), while Fv/Fm steadily decreased with increasing exposure time (Fig. 2). After 24 h the Fv/Fm values decreased from 0.76 to 0.64. Under 0.4 W/m^2 UV-B radiation, the oxygen evolution rates and Fv/Fm values gradually decreased with the extension of treatment, while Fv/Fm value seems to be more sensitive than oxygen evolution rate (Figs. 1 and 2). 0.8 W/m^2 UV-B radiation caused an 83.7% inhibition in oxygen evolution rate and Fv/Fm value decreased 87.3% after 24 h UV-B exposure, compared to the control group (Figs. 1 and 2). This result reflected that UV-B radiation damaged PSII reaction centers to oxidize water to product molecular O_2 and the photosynthesis process was almost destroyed. Hence, the effect of inhibition depended on UV-B radiation level and exposure time.

UV-B radiation reduced electron transport rate in the whole photosynthetic system: Fig. 3 indicated that rapid light curves of *Chlamydomonas reinhardtii* under different treated groups clearly showed distinctive shapes. Rapid light curves (RLC) are generated from the relative electron transport rate (rETR) responds to the different PAR irradiance during the rapid light curve steps (0, 32, 64, 192, 320, 448, 576, 704, 960, and 1216 μmol photons $m^{-2}s^{-1}$ PAR). The relative electron transport rate (rETR) is considered to be an approximation rate of electrons pumped through the electron transport chain; hence it reflects the photosynthetic activity. In the control group, the light curve of the control group steadily increased with incremental light intensity (PAR) ranging from 0 to 1216 μmol photons $m^{-2}s^{-1}$ and the maximum rETR was assumed to be much higher than 1200 μmol photo $m^{-2}s^{-1}$ (PAR). However, the light curve of the 0.8 W/m^2UV-B treatment group, reached a plateau at near 600 μmol photons $m^{-2}s^{-1}$ (PAR) and begun slightly declines, which was an obvious photoinhibition phenomenon and was a result of an imbalance rates between damage and the repair to photosystem.

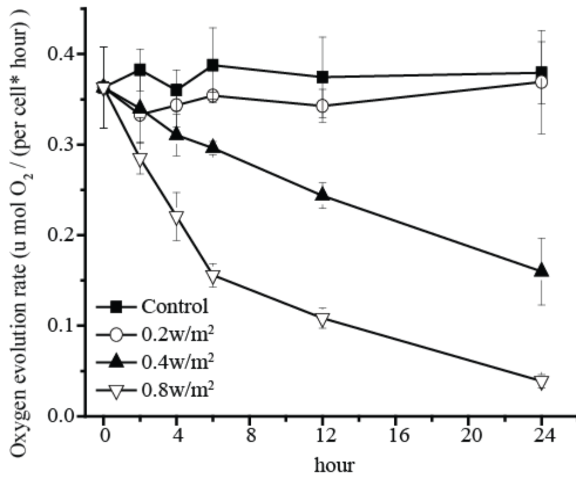

Fig. 1. Changes in photosynthetic activity (oxygen evolution rate) in *Chlamydomonas reinhardtii* exposed to different levels of UV-B radiation.

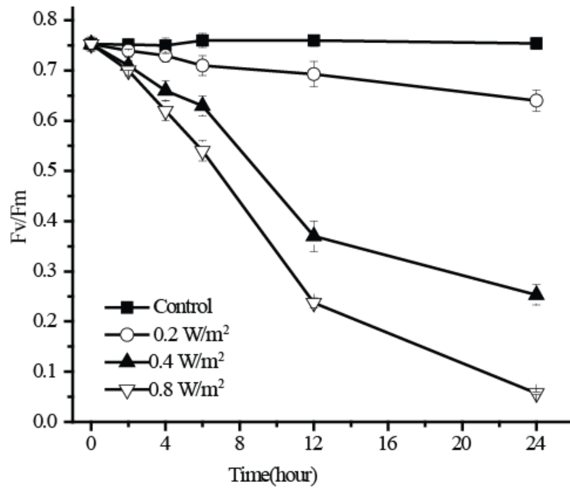

Fig. 2. Changes in photosynthetic activity (Fv/Fm) in *Chlamydomonas reinhardtii* exposed to different levels of UV-B radiation.

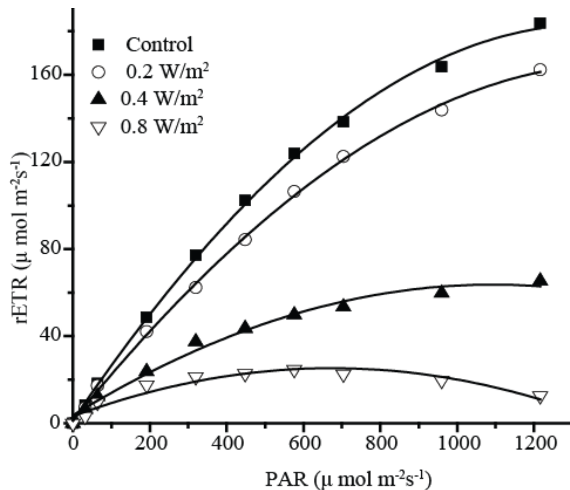

Fig. 3. The rapid light curves (RLC) of *Chlamydomonas reinhardtii* exposed to different levels of UV-B radiation.

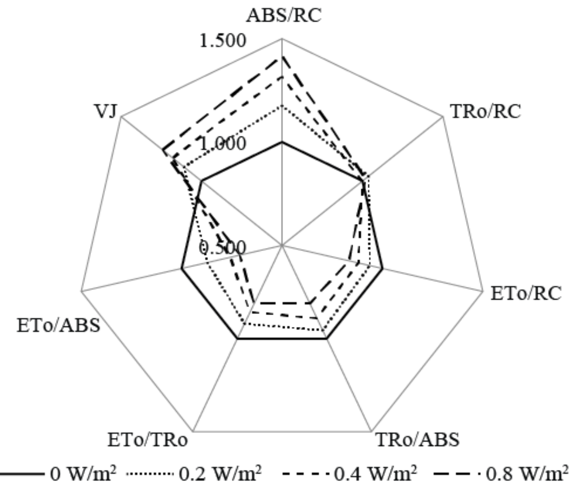

Fig. 4. Radar plots of chlorophyll a fluorescence parameters in *Chlamydomonas reinhardtii* exposed to different levels of UV-B radiation for 6 h.

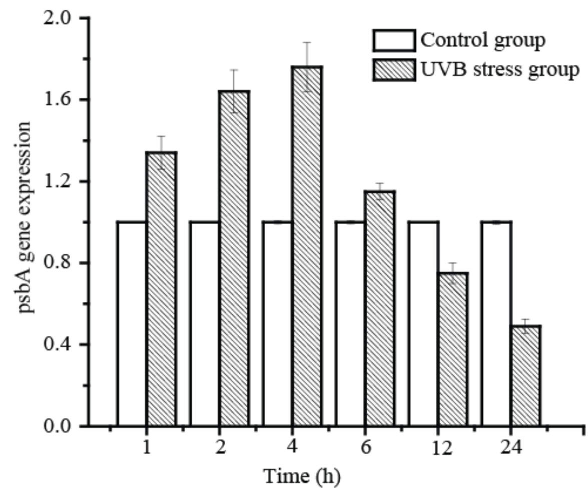

Fig. 5. psbA gene expression in *Chlamydomonas reinhardtii* exposed to 40 μW/ cm^2 UV-B radiation.

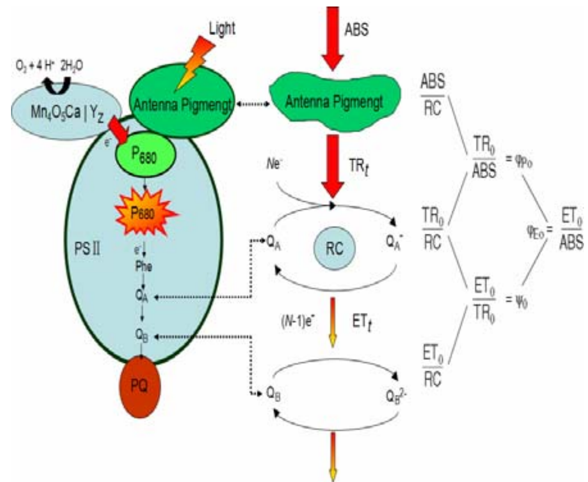

Fig. 6. A modified working model of the energy fluxes in the photosynthetic apparatus.

Table 2. Changes in photosynthetic rETRmax, Ik and α (alpha) in *Chlamydomonas reinhardtii* exposed to different levels of UV-B radiation.

UV-B (W/m^2)	0	0.2	0.4	0.8
rETRmax (μmol $m^{-2}s^{-1}$)	209.7 ± 18.9	158.3 ± 1.2	43.6 ± 9.2	17.6 ± 6.9
IK(μmol $m^{-2}s^{-1}$)	664.2 ± 49.4	582.6 ± 7.2	301.8 ± 36.7	238.2 ± 69.9
α	0.286 ± 0.008	0.272 ± 0.003	0.179 ± 0.003	0.082 ± 0.008

As showed in Table 2, the value of $rETR_{max}$ of the control group was significant different from all of the UV-B treated groups (0.2, 0.4 and 0.8 W/m^2). Hence the reduced value of $rETR_{max}$ of treated groups indicated that UV-B radiation caused some inhibition in the electron transport chain within thylakoid membranes, which result to reduce the activity of photosystem. Several derived parameters of rapid light curves, such as half-maximum photosynthesis (IK) and maximum photosynthetic efficiency (α), would further quantitatively illustrate the degree of photodamage caused by UV-B radiation. The control group had the highest IK value (664.2±49.4μmol $m^{-2}s^{-1}$) and α value (0.286±0.008), while the 0.8W/m^2 UV-B treatment group had the lowest value IK (238.2±69.9μmol $m^{-2}s^{-1}$) and α value (0.082±0.008) (Table 2).

UV-B radiation brought inhibition sites in the electron transport chain: Based on the analysis data from chlorophyll a fluorescence transient, the "JIP-test" has been developed for monitoring photosynthesis process under environment stress. "JIP-test" results can be showed as a spider-plot to present that the energy fluxes transfer though per reaction center (RC). Fig. 4 showed that different levels UV-B radiation significantly affected chlorophyll a fluorescence in *Chlamydomonas reinhardtii* after exposing for 6 hours. Compared with the control group, ET_O/TR_O, TR_O/ABS and ET_O/ABS of the 0.8 W/m^2 treated group decreased 16.9%, 19.3% and 17.8% respectively. In contrast to this repression, up-regulation of ABS/RC parameters was detected in 0.8 W/m^2UV-B group compared to control group. However, TRo/RC is no significant between all the groups.

UV-B radiation altered photosynthesis-related gene expression: The psbA gene encode D1 protein, which is a core subunit of the photosystem II providing Q_B-binding site. Under 0.4 W /m^2 UV-B exposure, the psbA gene expressions were strongly induced to up-regulated 37.1%, 62.4%, 78.3%, 18.3% folds compared with the control group after exposure for 1h, 2 h, 4h, and 6h, respectively (Fig. 5). But at 12, 24 h, the expressions of psbA gene were significantly repressed and reduced to75.0% and 49.1% of the control group respectively (Fig. 5).

Discussion

Many previous researches demonstrated the detrimental effects of UV-B radiation on organisms include dimerization of pyrimidine bases, membrane damage, protein destruction and hormone inactivation (Pfeifer & Besaratinia, 2012; Malloy *et al*.,1997; He & Häder, 2002a; Rastogi *et al*., 2010), and photosystem II is always the first and major target of UV radiation (Sangtarash *et al*., 2009; Wang *et al*., 2008; Wang *et al*., 2010b), but the mechanism of UV-B damage with more detailed information and the inhibition sites of UV-B radiation in the electron transport chain are still not clear.

Oxygen evolution rate is intimately related to photosynthesis activity and Fv/Fm reflects the potential open degree of the PSII reaction center (Hu *et al*., 2013). Our data clearly showed that UV-B radiation strongly impaired oxygen evolution and reduced photosynthetic efficiency (Figs. 1 and 2). Furthermore, those inhibitions depended on UV-B radiation level and exposure time. The similar reports were documented in barley (Carkirlar *et al*., 2008) and in algae (Franklin *et al*., 2003), which showed that UV-B radiation declined photosynthetic rate due to the reduction in Rubisco levels and adverse effect on electron transport under UV-B radiation (Carkirlar *et al*., 2008).

Rapid light curves (RLCs) reflect the physiological state of photosynthetic apparatus in PSII, and also provide detail information on the electron transfer process (Belshe *et al*., 2007). The fluorescence parameters (rETRmax, alpha and Ik) are derived from RLC, which can respectively reflect photosynthetic capacity (ETRmax), the photosynthetic efficiency (alpha) and tolerance to light (IK) during immediate short-term changes in PAR irradiance. We found that UV-B radiation diminished these derived fluorescence parameters (rETRmax, alpha and Ik) in *Chlamydomonas* (Table 2), which was consistent with other research in *Microcystis aeruginos*a (Yang *et al*., 2014). Fig. 3 indicated that UV-B radiation decreased ETRmax values, which implied that UV-B radiation impaired the photosynthetic electron transport chain and reduced electron transfer from light harvesting antenna to the whole PSII. Consequently, the less light energy was converted into chemical energy (ATP and NADPH) through electron transport chain, which could be seen from the lower photosynthetic efficiency (alpha) under UV-B radiation. It is noteworthy that photoinhibition occurred obviously at 0.8 W/m^2 UV-B group (Fig. 3) and corresponding IK value of this UV-B group dropped to 238.2μmol $m^{-2}s^{-1}$, which only accounts for 35.9% of the control group (Table 2). This result meant that the absorbed light energy by photosynthetic pigments exceeds utilization of energy in chloroplasts, which leads to decrease IK. The similar result also occurred in hybrid rice (Yu *et al*., 2013), *Althenia orientalis* (Karsten & Holzinger, 2012) and *Stylophora pistillata* (Itay *et al*., 2013). Therefore, it is reasonable to infer that UV-B radiation may block some sites in photosynthetic electron transport chain.

To more precisely understand the potential inhibition sites in the electron transport chain under UV-B radiation

stress, "JIP-test" was investigated to analyze photosynthesis process, which provides more detailed information about the energy fluxes per reaction center. Based on theory of the energy fluxes in the biomembrane, Strasser and his collaborators developed a highly simplified working model (Strasser *et al*., 2000) (as shown in Fig. 6). In this model, the photosynthesis started from the flux of photos (ABS) absorbed by antenna pigment (Chl). And then the energy was transferred to photosynthetic reaction centers (RC). In the reaction center (RC), some transferred energy was used to reduce the electron acceptor Q_A to Q_A^-, which was defined as trapping flux (TR). Similarly, electron transport (ET) referred to the energy flux that reoxidized Q_A^- to Q_A via forward electron transport to secondary quinone Q_B. Hence, the specific energy fluxes parameters (ABS/RC, TR/RC and TR/RC) were introduced to calculate energy fluxes and flux ratios within biomembranes. And those parameters respectively represented the energy absorbance (ABS), trapping (TR) and electron transport (ET) in per reaction center (RC). This simplified model could calculate the energy fluxes distribution and flux ratios from light absorption to electron transport chain in the photosynthetic apparatus. Hence it had been applied to various photosynthetic materials under different environment stresses (Strasser *et al*., 2000).

In this study, we found that there were significant increase in ABS/RC and no significant change in TRo/RC in algae after exposure 6 hours UV-B radiation (Fig. 4). ABS/RC represents electron transport distribution process from energy absorption by antenna pigments to the PSII reaction center (RC) and TRo/RC represents the maximal rate of an exciton trapped by the RC, leading to the reduction of Q_A to Q_A^-. Hence, the results of chlorophyll fluorescence parameters (ABS/RC and TRo/RC) implied that UV-B radiation did not affect the electrons transport from oxidation of water to pheophytin molecule (Phe), and subsequently passed on to reduce Q_A. These findings were also consistent with research in Cyanobacterium *Synechocystis*, where UV-B radiation does not affect the increased reduction of Q_A^- (Vass *et al*., 1999).

In contrast, there were the decrease values of ETo/TRo and ETo/ABS in algae under UV-B radiation (Fig. 4), which suggested that the electron transport flux (ET) became to diminish, and/or the less light energy could be continually transferred into the energy transport chain after passing Q_A. Thus the electron transfer step was seriously blocked from Q_A to Q_B. Vass also had observed the lower rates of electron transport between Q_A and Q_B, due to modification of the Q_B site (Vass, 1997). Moreover, the chlorophyll fluorescence parameters (V_J) would further support our result. Since V_J value represents the amounts of accumulated Q_A^-, UV-B radiation could significant increase V_J value in Fig. 6, which implied the electron was blocked in the downstream of Q_A.

Hence combining these results and the simplified working model (Fig. 6), we could draw a conclusion that UV-B radiation does not influence the electron transfers from light-induced oxidation of water to Q_A in the PSII, but can block the electron transport flux from Q_A to Q_B. So Q_B could be the primary inhibition site of photosynthetic electron transfer chain. Many studies demonstrated that the inhibition of electron transfer between Q_A and Q_B could frequently be observed under various unfavorable environment conditions as like salt stress (Strasser *et al*., 2000). Hence, the block of electron transport between Q_A and Q_B lead the decreased energy converts to chemical energy and finally reduces photosynthetic activity.

D1 proteins, the core proteins of PSII, maintain the stability of the PSII reaction center, and provide binding sites for redox cofactors of electron transport (include Q_B) (Teramoto *et al*., 2002). Many previous studies had demonstrated that D1 protein was quite sensitive to UV-B radiation and the expression of psbA gene (encode D1 protein) plays an important role in the repair UV-B-damage (Chaturvedi *et al*., 1998). To further analysis the connection between electron transport inhibitions sites in the Q_B and the loss of D1 proteins under UV-B radiation stress, the real time-PCR technology was applied to detect psbA gene expression. Our results with real-time PCR showed that the expression of psbA gene was excessive induced after 2, 4, and 6 h UV-B radiation exposure, which may be considered an adaptation response to UV-B radiation (Fig. 5). Since UV-B radiation induced degradation of D1 proteins can be repaired via the increasing psbA gene expression to synthesis newly D1 protein. The similar psbA gene expression also occurs at Cyanobacterium *Synechocystis* 6803 by low intensity UV-B stress (Mate *et al*., 1998), which can protect against UV-induced damage. Due to this efficient repair system, the damage induced by UV-B radiation becomes limited during the first 6 hours. However, the expression of psbA gene decreased after 12 and 24 h exposure to UV-B radiation, which implies that the photodamage induced by UV-B radiation (more than 12 hours) would not be repaired efficiently, and then UV-B radiation directly affected psbA gene transcription process and further inhibited D1 protein repair. Similar results could be obtained in pea using Northern blot (Mackerness *et al*., 1997) and Western blot in wheat exposed to UV-B (Chaturvedi *et al*., 1998).

Based on these results, it was clearly demonstrated that UV-B radiation reduced the photosynthesis activity and inhibited electron transport process. Our results also showed that UV-B radiation induced psbA gene expression, which implied that there was more damaged D1 protein and less normal D1 protein in PSII. Less normal D1 protein cannot provide enough binding sites for Q_B and then affected electron transport between Q_A and Q_B, which finally reduced photosynthetic activity. Therefore, the inhibition site of UV-B radiation on photosynthetic electron transport should be located in Q_B, and the damage of UV-B on D1 protein may be the cause of this inhibition.

Abbreviations: PAR, photosynthetically active radiation; PSII, photosystem II; Q_A, first plastoquinone electron acceptors; Q_B, second plastoquinone electron acceptors; RC, reaction center; RLC, Rapid light curves; rETR, relative electron transport rate.

Acknowledgements

The work was supported by Chinese Natural Science Foundation (30970688, 31370421), the National Major Programs of Water Body Pollution Control and Remediation (2014ZX07302003) and the Project of Chinese Manned Spaceflight.

References

Belshe, E., M. Durako and J. Blum. 2007. Photosynthetic rapid light curves (RLC) of *Thalassia testudinum* exhibit diurnal variation. *J. Exp. Mar. Biol. Ecol.*, 342: 253-268.

Carkirlar, H., N. Ciek, I. Fedina, K. Georgieva, A. Dogru and M. Velitchkova. 2008. NaCl induced cross-acclimation to UV-B radiation in four Barley (*Hordeum vulgare* L.) cultivars. *Acta Physiol. Plant.*, 30: 561-567.

Chaturvedi, R., R. Shyam and P. Sane. 1998. Steady state levels of D1 protein and psbA transcript during UV-B inactivation of photosystem II in wheat. *Iubmb Life,* 44: 925-932.

Franklin, L.A., C.B. Osmond and A.W. Larkum. 2003. Photoinhibition, UV-B and algal photosynthesis. In: *Photosynthesis in Algae. Advances in Photosynthesis and Respiration Vol. 14.* (Eds.): Larkum, A.W.D., S.E. Douglas and J.A. Raven Kluwer Academic Publishers, Dordrecht, pp. 351-384

Harris, E.H. 2001. *Chlamydomonas* as a model organism. *Ann. Rev. Plant Biol.*, 52: 363-406.

He, Y.Y. and D.P. Häder. 2002a. Involvement of reactive oxygen species in the UV-B damage to the cyanobacterium *Anabaena* sp. *J. Photochem. Photobiol. B: Biol.*, 66: 73-80.

He, Y.Y. and D.P. Häder. 2002b. UV-B-induced formation of reactive oxygen species and oxidative damage of the cyanobacterium *Anabaena* sp.: protective effects of ascorbic acid and N-acetyl-l-cysteine. *J. Photochem. Photobiol. B: Biol.*, 66: 115-124.

Hema, R., M. Senthil-Kumar, S. Shivakumar, P.C. Reddy and M. Udayakumar. 2007. *Chlamydomonas reinhardtii*, a model system for functional validation of abiotic stress responsive genes. *Planta,* 226: 655-670.

Hou, S.W., F. Zhou, S. Peng, H. Gao and X.D Xu. 2015. The HetR-binding site that activates expression of patA in vegetative cells is required for normal heterocyst patterning in *Anabaena* sp. PCC 7120. *Sci. Bull.*, 60: 192-201.

Hu, G., Y. Fan, L. Zhang, C. Yuan, J. Wang, W. Li, Q. Hu and F. Li. 2013. Enhanced Lipid Productivity and Photosynthesis Efficiency in a *Desmodesmus* sp. Mutant Induced by Heavy Carbon Ions. *PloS One,* 8: e60700.

Hu, J.L., X. Deng, N. Shao, G.H. Wang and K.Y. Huang. 2014. Rapid construction and screening of artificial microRNA systems in *Chlamydomonas reinhardtii*. *Plant J.*, 79: 1052-1064.

Itay, C., D. Gal, I. David and D. Zvy. 2013.UV-B as a photoacclimatory enhancer of the hermatypic coral *Stylophora pistillata*. *Open J. Mar. Sci.,* 3: 15.

Karsten, U. and A. Holzinger. 2012. Light, temperature, and desiccation effects on photosynthetic activity, and drought-induced ultrastructural changes in the green alga *Klebsormidium dissectum* (Streptophyta) from a high alpine soil crust. *Microbial Ecol.,* 63: 51-63.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using Real-Time Quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. *Methods*, 25: 402-408.

Longstaff, B.J., T. Kildea, J.W. Runcie, A. Cheshire, W.C. Dennison, C. Hurd, T. Kana, J.A. Raven and A.W. Larkum. 2002. An in situ study of photosynthetic oxygen exchange and electron transport rate in the marine macroalga *Ulva lactuca* (Chlorophyta). *Photosyn. Res.*, 74: 281-293.

Mackerness, S.A., B. Thomas and B.R. Jordan. 1997. The effect of supplementary ultraviolet-B radiation on mRNA transcripts, translation and stability of chloroplast proteins and pigment formation in *Pisum sativum* L. *J. Exp. Bot.*, 48: 729-738.

Malloy, K.D., M.A. Holman, D. Mitchell and H.W. Detrich. 1997. Solar UVB-induced DNA damage and photoenzymatic DNA repair in Antarctic zooplankton. *Proc. Natl. Acad. Sci.*, 94: 1258-1263.

Mate, Z., L. Sass, M. Szekeres, I. Vass and F. Nagy. 1998. UV-B-induced Differential Transcription of psbA Genes Encoding the D1 Protein of Photosystem II in the Cyanobacterium *Synechocystis* 6803. *J. Biol. Chem.*, 273: 17439-17444.

Merchant, S.S., S.E. Prochnik, O. Vallon, E.H. Harris, S.J. Karpowicz, G.B. Witman, A. Terry, A. Salamov, L.K. Fritz-Laylin and L. Mar Chal-Drouard. 2007. The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science,* 318: 245-250.

Mohajer, S., R.M Taha, M. Mohajer and I.Y. Javan. 2015. UV-B irradiation effects on biological activities and cytological behavior of Sainfoin (*Onobrychis viciifolia* Scop.) grown in vivo and in vitro. *Pak. J. Bot.*, 47: 1817-1824.

Pfeifer, G.P. and A. Besaratinia. 2012. UV wavelength-dependent DNA damage and human non-melanoma and melanoma skin cancer. *Photochem. Photobiol. Sci.*, 11: 90-97.

Ralph, P.J. and R. Gademann. 2005. Rapid light curves: a powerful tool to assess photosynthetic activity. *Aquat. Bot.*, 82: 222-237.

Rastogi, R.P, Richa, A. Kumar, M.B. Tyagi and R.P. Sinha. 2010. Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *J. Nucleic Acids*, 2010: 592980

Rastogi, R.P., R.P. Sinha, S.H. Moh, T.K. Lee, S. Kottuparambil, Y.J. Kim, J.S. Rhee, E.M. Choi, M. T. Brown, D.P. Häder and T. Han. 2014. Ultraviolet radiation and cyanobacteria. *J. Photochem. Photobiol. B: Biol.*, 141: 154-169.

Sangtarash, M.H., M.M. Qaderi, C. Chinnappa and D.M. Reid. 2009. Differential responses of two Stellaria longipes ecotypes to ultraviolet-B radiation and drought stress. *Flora-Morphol. Distribut. Funct. Ecol. Plant.*, 204: 593-603.

Sicora C., A. Szilard, L. Sass, E. Turcsanyi, Z. Mate and I. Vass. 2006. UV-B and UV-A radiation effects on photosynthesis at the molecular level. In: *Environmental UV Radiation: Impact on Ecosystems and Human Health and Predictive Models.*(Eds.): F. Ghetti, G. Checcucci and J.F. Bornman, Springer, Netherlands, pp. 121-135.

Sicora, C., Z. Mate and I. Vass. 2003.The interaction of visible and UV-B light during photodamage and repair of Photosystem II. *Photosynth. Res.*, 75: 127-137.

Sompornpailin, K. and S. Kanthang. 2015. Tobacco expressing PAP1 increases the responses to PAR and UV-A by enhancing soluble sugars and flavonoids and elevating plant protections. *Pak. J. Bot.,* 47: 595-602

Strasser, R.J. and A. Srivastava. 1995. Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. *Photochem. Photobiol.*, 61: 32-42.

Strasser, R.J., A. Srivastava and M. Tsimilli-Michael. 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: *Probing Photosynthesis: Mechanism, Regulation and Adaptation.* (Eds.): Yunus, M., U. Pathre and P. Mohanty, Taylor and Francis Press, London, pp. 445-483.

Teramoto, H., A. Nakamori, J. Minagawa and T.A. Ono. 2002. Light-intensity-dependent expression of Lhc gene family encoding light-harvesting chlorophyll-a/b proteins of photosystem II in *Chlamydomonas reinhardtii*. *Plant Physiol.*, 130: 325-333.

Turcsanyi, E. and I. Vass. 2000. Inhibition of photosynthetic electron transport by UV-A Radiation Targets the Photosystem II Complex. *Photochem. Photobiol.*, 72: 513-520.

Vass, I., D. Kirilovsky and A.L. Etienne. 1999. UV-B radiation-induced donor-and acceptor-side modifications of photosystem II in the cyanobacterium *Synechocystis* sp. PCC 6803. *Biochemistry*, 38: 12786-12794.

Vass, I., R.D. Szil and C. Sicora. 2005. Adverse effects of UV-B light on the structure and function of the photosynthetic apparatus. In: *Handbook of Photosynthesis* (Ed.): Pessarakli, M., (Second Ed.), CRC Press, Taylor & Francis Publishing Company, Florida, pp. 911-930.

Wang, G.H., K. Chen, L.Z. Chen, C.X. Hu, D.L. Zhang and Y.D. Liu. 2008. The involvement of the antioxidant system in protection of desert cyanobacterium *Nostoc* sp. against UV-B radiation and the effects of exogenous antioxidants. *Ecotoxicol. Environ. Safety,* 69: 150-157.

Wang, G.H., Z.J. Hao, R.H. Anken, J.Y. Lu and Y.D. Liu. 2010a. Effects of UV-B radiation on photosynthesis activity of *Wolffia arrhiza* as probed by chlorophyll fluorescence transients. *Adv. Space Res.,* 45: 839-845.

Wang, G.H., Z.J. Hao, Z.B. Huang, L.Z. Chen, X.Y. Li, C.X. Hu and Y.D. Liu. 2010b. Raman Spectroscopic analysis of a desert cyanobacterium *Nostoc* sp. in response to UV-B radiation. *Astrobiology*, 10: 783-788.

Yang, Z., F. Kong, X. Shi, Y. Yu and M. Zhang. 2014. UV-B radiation and phosphorus limitation interact to affect the growth, pigment content, and photosynthesis of the toxic cyanobacterium *Microcystis aeruginosa*. *J. Appl. Phycol.*, 26: 1669-1674.

Yoshida, K., E. Igarashi, E. Wakatsuki, K. Miyamoto and K. Hirata. 2004. Mitigation of osmotic and salt stresses by abscisic acid through reduction of stress-derived oxidative damage in *Chlamydomonas reinhardtii*. *Plant Sci.*, 167: 1335-1341.

Yu, G., W. Li, Z. Yuan, H. Cui, C. Lv, Z. Gao, B. Han, Y. Gong and G. Chen. 2013. The effects of enhanced UV-B radiation on photosynthetic and biochemical activities in super-high-yield hybrid rice Liangyoupeijiu at the reproductive stage. *Photosynthetica*, 51: 33-44.

(Received for publication 15 November 2015)