RESEARCH PAPERS

Physiological and ultralstructural changes of *Chlorella* sp. induced by UV-B radiation*

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Abstract In order to investigate the mechanisms of enhanced UV-B radiation on algae, the effects of UV-B radiation on the physiological and ultrastructural changes of *Chlorella* sp. were examined. The results showed that UV-B radiation could inhibit the growth and photosynthesis of microalgae. UV-B radiation at lower doses increased the photosynthetic pigment (chlorophyll a (Chla) and carotenoid (Car)) contents, while at higher doses of UV-B radiation Chla and Car contents were decreased. The ultrastructure of *Chlorella* sp. without exposure to UV-B showed that the thylakoid lamellae were clear and regular, the stroma of its chloroplast was apparent and clear. The globules with photosynthetic pigments and the cristae of mitochondria were clearly seen. After exposure to UV-B radiation at dose of 2.88 kJ/m^2, the thylakoid lamellae of *Chlorella* sp. were lost and dissolved, the globules which contained photosynthetic pigments in chloroplast were bleached; some mitochondria cristae were dissolved; slight plasmolysis was found in some *Chlorella* sp. cells. After exposure to 5.76 kJ/m^2 UV-B radiation, the thylakoid was in disarray and disintegration, plasmolysis was found in most cells, and the cell wall was broken and began to fall out. Many blank areas were observed in cells, mitochondria were seriously deformed and most of the mitochondria cristae were dissolved. Also, globules containing photosynthetic pigments in chloroplast were bleached and some empty globules were found in chloroplast. Therefore, UV-B radiation could damage cell structure of *Chlorella* sp., and this damage increased with the dose of UV-B radiation they exposed to.

Keywords: UV-B radiation, physiological changes, ultrastructure, *Chlorella* sp.

Increases in solar UV-B radiation (280—315 nm) reaching the Earth's surface due to stratospheric ozone depletion have raised concerns about UV-B impact on plants[1]. The marine phytoplankton, which is the base of the aquatic food chain, is very important on our planet and produces about the same biomass as all terrestrial plants taken together. Moreover, any changes to the size and composition of phytoplankton communities will directly affect food production for humans from marine sources. Another important role of marine phytoplankton is that the photosynthesis of phytoplankton can serve as a sink for atmospheric carbon dioxide. Phytoplankton populates the euphotic zone of the ocean and freshwater habitats where they receive sufficient solar radiation for photosynthetic processes. In addition to longer-wavelength radiation, phytoplankton is simultaneously exposed to solar UV-B radiation, which has been found to penetrate through most of the euphotic zone. Solar UV-B impairs the growth and reproduction of phytoplankton, their photosynthetic energy harvesting enzymes[2,3] and other cellular proteins, as well as photosynthetic pigment contents[4]. One of the major targets is the DNA, which strongly absorbs the short-wavelength solar radiation[5,6]. UV-B irradiation causes shifts in phytoplankton community structure, which may have consequences for the food web[7], resulting in reduced food production for humans and sink capacity for atmospheric carbon dioxide, as well as changes in species composition and ecosystem integrity[7].

Presently, the research on this topic is maily focused on the ecophysiological studies[8,9]. The effects of UV-B radiation on ultrastructure of marine microalgae have only been examined in a few species such as green alga[10], diatom[11], red alga[12]. The aim of this study is to observe the effects of UV-B radiation on the physiological and ultrastructural changes of *Chlorella* sp., and to investigate the mechanisms of enhanced UV-B radiation on algae and the adaptive mechanisms of algae to UV-B.

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1 Materials and methods

1.1 Algal species and cultural conditions

The *Chlorella* sp. provided by Marine Microalgae Research Center of Ocean University of China was cultured in Erlenmeyer flasks with f/2 medium[13], at the initial pH of 8.0±0.1 and the temperature of 20±1 ℃. The cultured *Chlorella* sp. were incubated under the condition of a dark: light cycle of 14: 10h with light intensity of 50μmol photon $m^{-2} \cdot s^{-1}$.

1.2 UV-B radiation

The source of UV-B radiation came from two Philips TL 40W UV-B tubes, which were covered by a film of cellulose acetate (0.12 mm) to remove all radiation below 280 nm. In order to minimize the change of the filter properties of the film, the cellulose acetate was presolarized for 48 h at a distance of 1 m from two UV-B tubes and changed weekly to ensure uniformity of UV-B transmission. The spectral irradiance from the tubes was determined with UV spectroradiometer (Beijing Normal University, Beijing) and only a thin layer of the algal suspension was used to ensure adequate UV-B penetration. The cells were exposed to UV-B rays at 3 W/m^2. The spectral irradiance was normalized at 300 nm to obtain UV-B_{BE} according to the formula of Caldwell[14]. After UV-B exposure for different time, both the treated and untreated groups were transferred into an illuminating incubator with dark: light of 14:10 and kept in standard culturing conditions for 48 h. All physiological experiments were carried out for them on triplicate. The control UV-B radiation was 0 kJ/m^2 UV-B dose, the treatments were 1.44 kJ/m^2, 2.88 kJ/m^2, 4.32 kJ/m^2, 5.76 kJ/m^2 and 7.2 kJ/m^2 UV-B doses, respectively.

1.3 Specific growth rate

Specific growth rate was calculated by the following formula: $\mu = (\ln N_2 - \ln N_1)/(t_2 - t_1)$, where N_2 and N_1 are the numbers of cells at t_2 and t_1 days at the exponential growth phase, respectively.

1.4 Assays of chlorophyll a (Chla) and carotenoids (Car) contents

According to the method of Jensen[15], samples were collected with glass filters (Whatman GF/F), then the filters were transferred to a 10 mL centrifuge tube with 90% acetone and kept overnight at 4 ℃. The extraction was performed and repeated until colorless. Measurements were performed with a spectrophotometer and the Chla and Car contents were calculated by Jensen's equations[15].

1.5 Measurement of photosynthetic rate

The measurement of photosynthetic rate was carried out by the method of Gao et al.[16].

1.6 Ultrastructure observation

The alga samples were taken from the suspensions and exposed to UV-B for 3 days, then harvested by centrifugation. The pellet obtained was first fixed for 3 h at room temperature with 2.5% glutaraldehyde (pH 7.3), followed by the second fixation with 1% osmium tetroxide for 1 h at room temperature. The samples were then dehydrated through an ethanol series and embedded in Spurr resin. The ultra-thin sections were cut by a microtome (LKB-V). The sections were stained with uranyl acetate and lead citrate, and examined under a transmission electron microscope (HITACHI H-7000).

1.7 Statistical analysis

Statistical differences between UV-B radiation treated and untreated groups were determined by t-test.

2 Results

2.1 Effects of UV-B radiation on the growth of *Chlorella* sp.

The growth of *Chlorella* sp. was inhibited by UV-B exposure and with the increasing of UV-B radiation doses this inhibition was more obvious (Fig. 1). When UV-B radiation dose was at the level of 1.44 kJ/m^2 the growth rate decreased by 11.0%, which is statistically significant when compared with the control ($p < 0.05$).

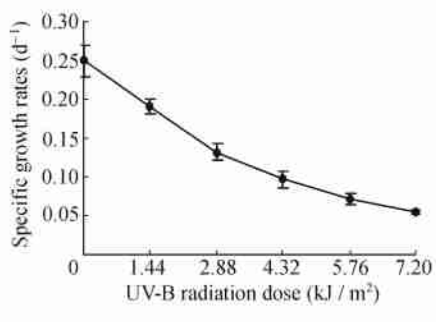

Fig. 1. Effects of UV-B radiation on growth of *Chlorella* sp.

2.2 Effects of UV-B radiation on Chla and Car contents of *Chlorella* sp.

The effects of UV-B radiation on Chla and Car contents of *Chlorella* sp. are shown in Fig.2. When *Chlorella* sp. was exposed to UV-B radiation at a relatively low dose (1.44 kJ/m^2), the photosynthetic pigment contents increased by 8.65% for Chla ($p<0.05$) and by 5.33% for Car ($p>0.05$), respectively. Chla and Car contents began to decrease when the UV-B radiation was higher than 1.44 kJ/m^2. After exposed to 7.2 kJ/m^2 UV-B radiation, the Chla and Car contents of *Chlorella* sp. decreased by 20.2% ($p<0.01$) and 21.1% ($p<0.01$), respectively, when compared to the control.

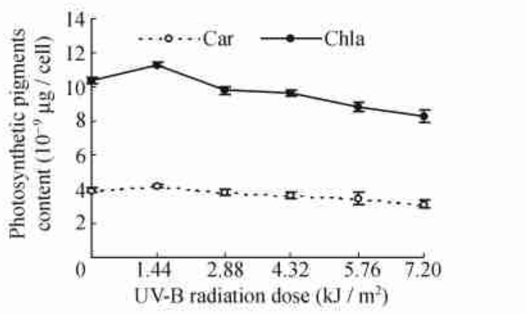

Fig. 2. Effects of UV-B radiation on photosynthetic pigments contents of *Chlorella* sp.

2.3 Effects of UV-B radiation on photosynthetic rate of *Chlorella* sp.

Fig. 3 shows that the photosynthetic rate of *Chlorella* sp. decreases with the increase of UV-B radiation doses, showing an inhibitory effect of UV-B on photosynthesis of microalgae. When UV-B radiation doses were at the levels between 0 and 4.32 kJ/m^2, photosynthetic rate decreased sharply, but when the UV-B radiation doses were at the levels between 4.32 and 7.2 kJ/m^2, photosynthetic rate remained at such a low rate. When UV-B radiation dose was at the level of 1.44 kJ/m^2, photosynthetic rate decreased by 49.6%, which was significantly different from that of the control group ($p<0.01$).

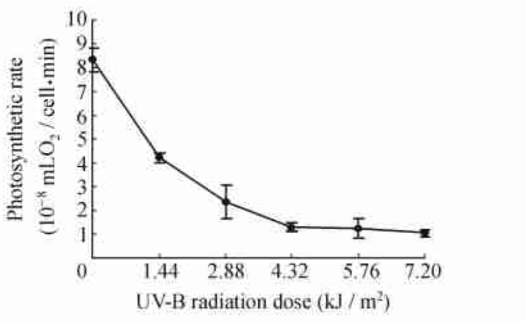

Fig. 3. Effects of UV-B radiation on photosynthetic rate of *Chlorella* sp.

2.4 Ultrastructure of *Chlorella* sp. cells

Under the electron microscope, the unradiated *Chlorella* sp. cells were spherical or oval in shape, with a cup-shaped or marginal babularis chloroplast, which occupied about one third of the total volume of the cell (Fig.4 (a)). Thylakoid lamellae with regular arrangement were vivid. The stroma in chloroplast was transparent and clear. Some globules containing photosynthetic pigments existed in chloroplasts (Fig.4 (a)). The mitochondria with an inner and an outer membrane were very big and their cristae were clearly visible (Fig.4 (b)), and nucleus and nucleolus could be also observed (Fig.4 (c)). No Golgi and pyrenoid was found in the *Chlorella* sp. cells.

After exposed to UV-B radiation of 2.88 kJ/m^2, the cell wall of *Chlorella* sp. was thicker than that of the unradiated *Chlorella* sp. cells. The thylakoid lamellae disappeared or disintegrated (Fig.4 (d), (e)). The globules containing photosynthetic pigments in thylakoid were bleached (Fig.4 (e)). The mitochondria cristae were partly dissolved, and slight plasmolysis was found in some *Chlorella* sp. cells (Fig.4 (d), (e)).

After exposed to UV-B radiation at the dosage of 5.76 kJ/m^2, the cell wall of *Chlorella*. sp became thicker than that of the control ones. The arrangement of thylakoid was in disorder and disintegration (Fig.4 (f)). The stroma in the cells was very thick, and some of them are aggregated into one big block, showing a very high electronic density (Fig.4 (g), (h)). The mitochondria became deformed and most of their cristae were dissolved; serious plasmolysis was found in some *Chlorella* sp. cells (Fig.4 (i)); some cell walls were broken and began to fall out (Fig.4 (j)); many blank areas were found in *Chlorella* sp. cells; the globules containing photosynthetic pigments in thylakoid were bleached and some blank walls of globules were observed in chloroplasts (Fig.4 (f)). Stroma in nucleus was thick and the electronic density was very high. Although the intracellular structure was seriously damaged, the nucleolus remained unchanged, meanwhile binary fission was found in one of the *Chlorella* sp. cells (Fig.4 (g)).

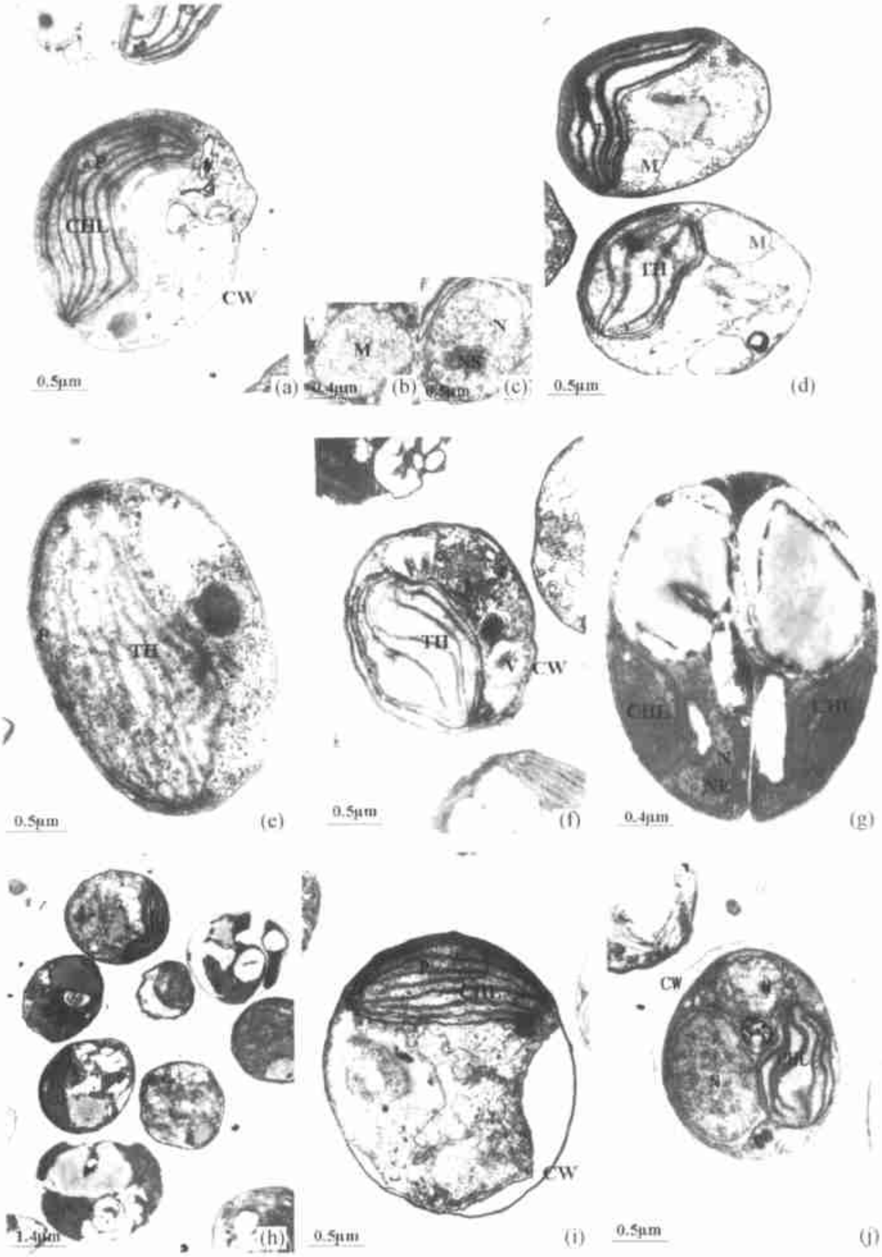

Fig. 4. The ultrastructure of UV-B treated *Chlorella* sp. cells. (a)—(c) The ultrastructure of *Chlorella* sp. cells in control experiment. (a) The stroma of chloroplast (CHL) was apparent and clear, the thylakoid (TH) lamellae were clear; the arrangement was regular. (b) Mitochondria (M). (c) Nucleus (N). (d)—(e) the ultrastructure of *Chlorella* sp. cells in 2.88 kJ/m^2 UV-B treated experiment. (d) Cell wall (CW) became thicker; thylakoid (TH) lamellae were lost and dissolved; some mitochondria (M) cristae were dissolved; slight plasmolysis was found in some *Chlorella* sp. cells. (e) the globules containing photosynthetic pigments (P) in chloroplast were bleached. (f)—(j) the ultrastructure of *Chlorella* sp. cells in 5.76 kJ/m^2 UV-B treated experiment. (f) cell wall (CW) became thicker; the arrangement of thylakoid (TH) was in confusion and disintegration; plasmolysis was found in most of *Chlorella* sp. cells. (g) binary fission was found in one *Chlorella* sp. cell. (h) many blank areas were found in the *Chlorella* sp. cells. (i) mitochondria (M) were seriously deformed and most of the mitochondria cristae were dissolved; globules containing photosynthetic pigments (P) in chloroplast were bleached. (j) cell wall (CW) was broken and began to fall out.

3 Discussion

Our studies indicated that UV-B radiation inhibited growth of marine microalgae, which is a phenomenon also found by other investigators. Masi et al.[17] reported that cell division rate in the UV-B-treated samples was slower than that in the control samples. The UV-B-induced inhibition of growth rate might be due to an inhibition of cell division, possibly by interfering with protein synthesis in G_1 or G_2 of the cell cycle[18].

In our experiment, UV-B radiation decreased the photosynthetic rate of *Chlorella* sp., which is consistent with the results of Lesser[19]. Many studies have shown that UV-B radiation reduced the photosynthesis of phytoplankton[20,21]. In UV-B sensitive algae, photosynthetic capacity may be reduced directly by the effect of UV-B radiation on photosynthetic enzymes or disruption of PS Ⅱ reaction centers, or indirectly by effects on photosynthetic pigments[22].

An increase of Chla and Car contents was found in *Chlorella* sp. by our experiments at relatively low UV-B dosage (1.44 kJ/m^2). This contradicts with numerous studies in which UV-B caused decreases in chlorophylls or carotenoids[23]. On the other hand, Döhler et al.[24] showed an increase in the Chla content of diatoms exposed to low UV-B doses. The increase of Car content suggests that when the UV-B doses were relatively low, the antioxidants in *Chlorella* sp. were induced to elevate to defend against UV-B radiation. In our study, Car content was increased at the level of 1.44 kJ/m^2 to remove reactive oxygen species (ROS). Chla and Car contents decreased with increasing UV-B doses at relatively high UV-B doses, which is consistent with Agrawal's results[25], who found that UV-B caused a decrease in photosynthetic pigment (particularly Chla) in the green algae *Chlorococcum infusionum* and *Chlorogonium elongatum*. We speculate that the reason for the decrease of Chla and Car contents might be that when the UV-B doses were relatively high the D1 protein of PS Ⅱ was destroyed and electron transfer was hampered.

An increase of starch content in chloroplast after UV-B exposure as Malanga et al.[26] described for *Chlorella vulgaris* was not observed in our study. We found that chloroplast was influenced most in all organelles of a *Chlorella* sp. cell. After being exposed to UV-B radiation, thylakoid lamellae were changed, the globules containing photosynthetic pigments in thylakoid were bleached, so that photosynthesis was damaged. These results are consistent with the reduced photosynthetic rate demonstrated in this experiment. Algal pigments mainly include photosynthetic, UV-shielding, phytochrome and respiratory pigments, and UV-B mainly damages photosynthetic pigments. Enhanced UV-B radiation could significantly reduce chlorophyll. Chlorophyll a is more easily damaged than chlorophyll b; while carotenoids and phycocyanin are more resistant to UV-B radiation[27].

Mitochondria, the center of respiration and energy-yielding as well as the energy source of alga growth, takes part in kinds of synthetic reactions, uptaking of inorganic nutritive elements and transportation of assimilation products. At the same time, it is the energy source of cell; therefore its size and number reflect endocellular respiration level. Our experiment showed that mitochondria cristae in *Chlorella* sp. cells were partly dissolved after the cells were exposed to UV-B radiation of 2.88 kJ/m^2 and further damaged after exposure to UV-B radiation of 5.76 kJ/m^2, which suggested that the respiration intensity attenuated so that the intracellular metabolic activity reduced. The increase in cell wall thickness was an adaptive response to UV-B radiation. Higher plants can form some defensive mechanisms against UV-B radiation, such as changes of morphologic structure, an increase in leaf blade stroma and epiderm thickness, and so on, to defend or attenuate UV-B radiation penetrating into cells and damage organelle so as to damage plants[28]. Our results showed that the damage of *Chlorella* sp. cell structure became more obvious with the increase of UV-B radiation dose. Poppe et al.[12] found that after 6 and 8 hours of artificial UV-exposure a vesiculation of the chloroplast thylakoids and changes in the membrane structure of mitochondria were observed, which disappeared after 12 h of UV treatment. No significant changes were detectable compared to the control ones after 12—16 h of UV exposure. In our study, after being treated with UV-B radiation of 2.88 kJ/m^2, the cell structure of *Chlorella* sp. was changed slightly, but when the *Chlorella* sp. cell was treated by UV-B radiation of 5.76 kJ/m^2, the cell structure changed remarkably. We speculate that the structure could be recovered after removal of UV-B radiation at lower dosage, but not at higher dosage. There was a dose-effect relationship in the damage of UV-B radiation to ultrastructure of *Chlorella* sp. cells.

From our results, we assume that the mechanisms of how UV-B radiation affacts algae might be as follows: Firstly, UV-B radiation damages DNA and causes a lowering of D1 and D2 proteins. In this case, under enhanced UV-B radiation, *Chlorella* sp. cells operate with a smaller number of PS Ⅱ reaction centers than control cells, so that the photosynthetic rate and growth rate significantly decreased. Secondly, the ultrastructure of *Chlorella* sp. was damaged. When the UV-B doses were relatively low, the cell wall of *Chlorella* sp. was thickened to protect the cell. When the UV-B doses were relatively high, the cell wall was broken and fell out, so the cytoplasm was leaked out and the cell ultrastructure was greatly damaged, then the cell division rate reduced and the growth rate lowered.

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