Ultraviolet irradiation induced oxidative stress and response of antioxidant system in an intertidal macroalgae *Corallina officinalis* L.

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Abstract

The response of the antioxidant defense system of an intertidal macroalgae *Corallina officinalis* L. to different dosages of UV-B irradiation was investigated. Results showed that superoxide dimutase (SOD) and peroxidase (POX) increased and then maintained at a relatively stable level when subjected to UV-B irradiation. Catalase (CAT) activity under medium dosage of UV-B irradiation (Muv) and high dosage of UV-B irradiation (Huv) treatments were significantly decreased. Ascorbate peroxidase (APX) activity first remained unaltered and then increased in Huv treatment. In addition, the assay on isozymes was carried out using non-denaturing polyacrylamide gel electrophoresis (PAGE). The activities of some SOD isoforms were altered by UV-B. Two new bands (POX V and POX VII) appeared upon exposure to all three UV-B dosages. CAT III activity was increased by low dosage of UV-B irradiation (Luv), whereas CAT III and CAT IV disappeared when the alga was exposed to Muv and Huv. Two bands of APX (APX VI and APX VII) were increased and a new band (APX X) was observed under Huv exposure. \( \text{H}_2\text{O}_2 \) and thiobarbituric acid reacting substance (TBARS) increased under Muv and Huv treatments. Overall, UV-B protection mechanisms are partly inducible and to a certain extent sufficient to prevent the accumulation of damage in *C. officinalis*.

Key words: UV-B irradiation; *Corallina officinalis* L.; superoxide dimutase; catalase; ascorbate peroxidase; peroxidase

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Introduction

Depletion of stratospheric ozone has led to an increase in solar UV-B radiation (280–320 nm) reaching the earth’s surface (Kerr and McElroy, 1993; Caldwell et al., 1998). The process of ozone depletion is expected to increase and spread to a broader range of altitudes and latitudes during this century (Tabazadeh et al., 2000). According to calculations based on a global climate model, the most severe depletions of stratospheric ozone will occur during 2010 to 2019 (Shindell et al., 1998). Enhanced exposure to UV-B radiation is potentially detrimental to all forms of life, but particularly to all photosynthetic organisms due to their requirement for light (Sinha et al., 2003). The intertidal zone is characterized by large environmental variations due to the alternation of emersion and immersion phases. The seaweed species live in the transition zone, which inevitably exposes them to aquatic and aerial climatic regimes, thus they must be adapted to short-term environmental variations over 12-hr tidal cycles. At emersion, seaweed is exposed to direct sunlight and higher UV-B radiation than when immerged, therefore, enhanced UV-B radiation at earth’s surface may directly affect the growth, vertical stratification and communities of seaweeds (Bischof et al., 1998; Makarov and Voskoboinikov, 2001; Johansson and Snoeij, 2002; Roleda et al., 2006b). UV tolerant species populate the tidal zone, while more sensitive species are found in deeper waters (Roleda et al., 2005). Studies suggested that the influence of UV-B at the ecosystem level may be more pronounced on community and trophic level structure, and hence on subsequent biogeochemical cycles, than on biomass levels per se (Häder et al., 2007).

Exposure to UV-B leads to an generation of activated oxygen species (AOS) such as singlet oxygen (\( \text{O}_2^* \)), superoxide (\( \text{O}_2^- \)), hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) and hydroxyl radicals (-OH) (Moldau, 1999). High AOS leads to an oxidative destruction of cell components through oxidative damage of nucleic acid, membrane lipids, protein and enzymes (Davis, 1987; Imlay and Linn, 1998; Aguilera et al., 2002; Shiu and Lee, 2005; Roleda et al., 2006a, 2006b). An efficient antioxidant defense system have developed in plants to counteract oxidative stress, which includes superoxide dimutase (SOD; EC1.15.1.1), catalase (CAT; EC1.11.1.6), peroxidase (POX; EC1.11.1.7), ascorbate peroxidase (APX; EC1.11.1.11), glutathione reductase (GR; EC1.6.4.2), dehydroascorbate reductase (DHAR; EC1.8.5.1), and monodehydroascorbate reductase (MDHAR; EC1.6.5.4). In plants, the generated \( \text{O}_2^- \) can be...
No. 5 Ultraviolet irradiation induced oxidative stress and response of antioxidant system in an intertidal macroalga Corallina officinalis L.  717

converted into H₂O₂ and O₂ by several SOD isoenzymes: mitochondrial manganese SOD (Mn-SOD), chloroplast iron SOD (Fe-SOD) and cytosolic copper and zinc SOD (Cu/Zn SOD) (Getzoff et al., 1989). CAT and POX efficiently catalyze the breakdown of H₂O₂. APX is another powerful H₂O₂ scavenging enzyme, which utilizes AsA to eliminate the toxic product H₂O₂ by the oxidation of AsA to the monodehydroascorbate (MDHA) (Mittler, 2002). APX isozymes are distributed in at least four distinct cellular compartments, including stromal APX (sAPX), thylakoid membrane-bound APX (tAPX) in chloroplasts, microbody membrane-bound APX (mAPX) and cytosolic APX (cAPX) (Asada, 1992).

There is significant evidence showing that alga exposed to oxidative stress tends to increase the activities of ROS scavenging enzymes (Malanga et al., 1997; Huang et al., 2002; Rijstenbil, 2002; Rossa et al., 2002; Shiu and Lee, 2005). This indicates that higher and more stable antioxidant enzyme activities, either constitutive or induced, are associated with a higher stress tolerance in algae. The comparison of antioxidant enzyme activities among twenty-two macroalgal species (five green, seven red, and ten brown) to UV radiation showed that algal tolerance to oxidative stress was correlated with an enhanced oxygen-reactive scavenging system (Aguilera et al., 2002). It is a reasonable conclusion that the antioxidant defense mechanism against ROS is pivotal for algal survival under stressful conditions (Collén and Davison, 1999; Aguilera et al., 2002; Shiu and Lee, 2005). In our previous study, the deleterious effect of UV-B on the growth of two species of marine algae was found, but its effect changed in relation to the dosage of UV-B (Li et al., 2008). It is hypothesized that the defense system of different algae possess dissimilar initial activity, especially different sensitivity and irritability responses to UV-B stress. However, there has been little systematic research into the chain of events causing the induction of specific isoforms of antioxidant enzymes in intertidal macroalgae against varying UV-B dosages.

In the present study, responses of antioxidants and enzymes were investigated in detail. We evaluated the performance of SOD, POX, CAT and APX activities and isoforms in Corallina officinalis L., which catabolized O₂⁻ and hydrogen peroxide, to further identify the biochemically relevant pathways and protective mechanisms when exposed to UV-B.

1 Materials and methods

1.1 Algal materials and growth conditions

C. officinalis used was collected from Taiping Angle of Qingdao, China in July 2007. Fresh algal material was washed repeatedly after sampling. The alga was cultured in an aquarium filled with f/2 medium (Guillard and Ryther, 1962), while fresh air was provided continuously by pump. The culture was kept at 25°C/20°C in the day and night with a 12-h photoperiod under an illumination intensity of 70 μmol photons/(m²·sec) PAR provided by 30 W cool-fluorescent lamps.

1.2 UV-B exposure treatment

UV-B irradiation (also containing UV-A) was provided by eight UV fluorescent lamps (Philips TL 40 W/12 μV, The Netherlands) covered with a filter of cellulose diacetate (0.12 mm) to filter UV-C (< 280 nm) irradiation, with its maximum emission appearing at 312 nm. In order to minimize potential change of the filter properties of the film, the cellulose diacetate was continuously presolarized for 72 hr. Cellulose acetate filters used in the equipment were replaced weekly to ensure the uniformity of irradiation. The radiant intensity was measured by UV-B spectroradiometer (Beijing Normal University, China) and normalized to obtain the biological effective (BE) UV-B irradiation (UV-B_{BE}) according to the formula established by Caldwell (1971). In this experiment, the doses of UV-B were 1.6 kJ/(m²·day) (low UV-B dosage, Luv), 4.8 kJ/(m²·day) (medium UV-B dosage, Muv), and 9.6 kJ/(m²·day) (high UV-B dosage, Huv). The alga was exposed to UV-B for 8 hr per day (09:00–17:00), and the group without UV-B radiation was used as the control. This experiment lasted for 12 days.

1.3 Hydrogen peroxide measurement

H₂O₂ was determined according to Alexieva et al. (2001). The alga (0.2 g) was homogenized in an ice bath with 3 mL of 0.1% TCA. The homogenate was centrifuged at 12,000 r/min for 15 min, and then the 0.5 mL supernatant was mixed with 0.5 mL of 10 mmol/L potassium phosphate buffer (pH 7.0) and 1 mL of 1 mmol/L KI. The absorbance was measured at A₅₉₀. The content of H₂O₂ was determined using a given H₂O₂ standard curve.

1.4 Thiobarbituric acid reacting substance (TBARS) determination

TBARS content was determined based on the method described by Heath and Packer (1968). The alga (0.2 g) was homogenized in an ice bath with 3 mL of 0.1% TCA. The homogenate was centrifuged at 10,000 r/min for 10 min. Then 0.5 mL supernatant was mixed with 2 mL of 20% TCA including 0.5% TBA. After heating at 100°C for 30 min, the reaction mixture was centrifuged at 15,000 r/min for 15 min. The TBARS contents were calculated based on A₅₃₂–A₆₀₀ with the extinction coefficient of 155 mmol/cm.

1.5 Enzyme extraction and assay

The algal tissues (0.5 g) were homogenized in 3 mL extraction buffer containing 50 mmol/L phosphate buffer (pH 7.0), 1 mmol/L EDTA, and 1% PVP. The homogenates were centrifuged at 12,000 r/min for 10 min and the supernatant was prepared for the determination of the activities of SOD, POX, and CAT. APX was extracted in a buffer including 50 mmol/L phosphate buffer (pH 7.0), 1 mmol/L EDTA, 1% PVP and 2 mmol/L AsA, then centrifuged at 12,000 r/min for 10 min. All steps in the preparation of the enzyme extract were carried out at 0–4°C.
The SOD activity was determined based on its capacity to inhibit reduction of nitroblue tetrazolium using the method of Giannopolitis and Ries (1977). The POX activity was determined by measuring the increase in absorption at $A_{470}$ due to the formation rate of tetraguaiacol ($\varepsilon$: 26.6 mmol/cm) according to Dias and Costa (1983). The CAT activity was measured by estimating the decreasing rate of ascorbate oxidation at $A_{240}$ ($\varepsilon$: 40 mmol/cm) by the method of Rao et al. (1996). The APX activity was measured by estimating the decreasing rate of ascorbate oxidation at $A_{290}$ ($\varepsilon$: 2.8 mmol/cm) according to Nakano and Asada (1981).

1.6 Protein determination

Protein concentration was evaluated by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

1.7 Native PAGE and activity staining

Enzyme extracts of the alga were subjected to non-denaturing conditions in 8% discontinuous PAGE, according to the method of Laemmli (1970) with some modifications. Electrophoretic separation was performed with constant current of 20 mA/gel at 4°C. Staining of SOD isozymes was fulfilled as following: the gels were incubated in 0.25 mmol/L NBT for 20 min, which were replaced by 8 mmol/L EDTA and 0.05 mmol/L riboflavin for 20 min in the dark. Then the gel was exposed to an intense white light until SOD isoenzymes bands could be observed clearly on dark blue background. The SOD isoforms were further classified by their differential sensitivity to KCN and H$_2$O$_2$ by the method of Beauchamp and Fridovich (1971) with some modifications. The POX isoenzymes were visualized by the incubation of gels in a solution consisting of 200 mmol/L acetate buffer (pH 5.0), 0.3% H$_2$O$_2$ and 2 mmol/L benzidine, which were appeared with brown bands after about 20 min (Van Loon, 1971). Staining of CAT isoenzyme was based on the method of Woodbury et al. (1971). Gels were incubated in 3.3 mmol/L H$_2$O$_2$ for 25 min and developed in a 1% FeCl$_3$ and 1% K$_3$Fe(CN)$_6$ solution until green color appeared on the gels. Detection of APX was subjected to native PAGE as described above, except that the carrier buffer contained 2 mmol/L ascorbate (AsA) to maintain the activity of APX isoenzyme. The gels were submitted to a pre-run for 30 min to allow ascorbate to enter in the gel. Staining of APX isozymes was by the method of Mittler and Zilinskas (1993). The gels were incubated in 50 mmol/L sodium phosphate buffer (PBS, pH 7.0) with 2 mmol/L AsA for 30 min. After incubating with 50 mmol/L PBS (pH 7.0) containing 4 mmol/L AsA and 2 mmol/L H$_2$O$_2$ for 20 min, the gels were submerged in a solution of 50 mmol/L PBS (pH 7.8) including 2.5 mmol/L nitroblue tetrazolium and 30 mmol/L N,N,N,N-tetramethylethylenediamine for about 10 min. Then APX bands were visible against a blue background.

1.8 Data statistics

Values in the text are presented as mean ± SD. The determinations of enzyme activity were performed in three independent experiments. All data were analyzed by one-way ANOVA, and significant differences from the control values were calculated by Duncan’s multiple range test at $P < 0.05$. 

![Fig 1](chart.png)  
Fig. 1 Effect of UV-B on SOD (a), CAT (b), POX (c) and APX (d) activities of C. officinalis. ck: control without UV-B; Luv: low level of UV-B at 1.6 kJ/(m$^2$·day); Muv: medium level of UV-B at 4.8 kJ/(m$^2$·day); Huv: high level of UV-B at 9.6 kJ/(m$^2$·day). Results are expressed as means ± SD ($n=3$). *, **, significantly different from ck at $P < 0.05$ and $P < 0.01$, respectively.
2 Results

2.1 Activities of antioxidant enzymes

The different enzyme activities of the alga were observed after exposure to different UV-B dosages. According to Fig. 1a, Luv treatment was effective in increasing SOD activity, which increased about 42.5% comparing with the control. However, SOD activity remained unchanged under Muv and Huv treatments. Low dose of UV-B failed to affect CAT activity, but Muv and Huv treatments significantly inhibited CAT activities (Fig. 1b). The activity of POX was affected by Luv exposure, but which decreased gradually under medium and high dosages of UV-B comparing with the control (Fig. 1c). APX activities in both Luv and Muv treatments had no significant change comparing with the control. However, it increased under the Huv treatment by the end of exposure. (Fig. 1d).

2.2 TBARS and H$_2$O$_2$ concentration

TBARS concentrations increased remarkably at the end of the UV-B exposure when subjected to Muv and Huv, but there was no significant effect in Luv treatment in comparison with the control (Fig. 2a).

UV-B induced H$_2$O$_2$ accumulation in the algal tissues, especially under the Muv and Huv treatments. H$_2$O$_2$ concentrations were 33.5% and 55.6% higher than the control value under Muv and Huv treatments, respectively (Fig. 2b). However, H$_2$O$_2$ concentration did not change markedly in Luv tissues comparing with the control.

2.3 Activity of enzyme isoenzymes when subjected to UV-B

In Fig. 3a, SOD presented nine bands. Compared with the control, SOD III, SOD IV, SOD V and SOD IX activities all increased significantly while SOD VII and SOD VIII activities decreased to a certain extent under the treatment of Luv. SOD VII and SOD VIII activities were reduced by Huv exposure. SODI almost disappeared in Muv and Huv treatments. The control showed four CAT isoforms (CAT I, CAT II, CAT III and CAT IV). CAT III activity increased significantly under Luv treatment, while CAT IV disappeared. Both CAT III and CAT IV were eliminated when the alga was exposed to Muv and Huv treatments (Fig. 3b). Compared with the control, POX I, POX II, POX IV and POX VI showed similar activities, whereas two new bands (POX V and POX VII) appeared upon exposure to UV-B. Only POX III activity decreased remarkably in all three treated algal materials (Fig. 3c). APX isoenzymes also represented mixed results. As illustrated by Fig. 3d, APX V activity increased under all three UV-B doses; however, APX IX slightly decreased under three treatments when compared with the control. In addition, APX VI and APX VII significantly increased and a new band (APX X) was unexpectedly observed under Huv treatment.

3 Discussion

In addition to its function as a toxic metabolite, H$_2$O$_2$ is also a signaling molecule that mediates the response of plants to both biotic and abiotic stresses (Vanlerberghe and McIntosh, 1996; Van Breusegem et al., 2001). In our
study, H$_2$O$_2$ concentrations increased slightly and rapidly, and SOD and POX activities effectively increased in Luv treatment (Figs. 2b, 1a, and 1c). H$_2$O$_2$ mediated the regulation of transcription in response to UV-B exposure as an important early upstream signal (Brosché and Strid, 2003). Shi and Lee (2005) also found that UV-B activates the production of O$_2^-$ and H$_2$O$_2$ for triggering the antioxidant defence system in Ulva fasciata. This suggests that H$_2$O$_2$ may act more as a signal molecule than directly inducing oxidative damage at lower UV-B dose. Our results are consistent with these findings. Furthermore, H$_2$O$_2$ increased significantly under Muv and Huv treatments at the end of UV-B exposure (Fig. 2b). Although the activity of APX was modified by Huv treatment (Figs. 1d), the relatively stable activity of SOD and POX had very similar values to that of the control, and significantly decreased CAT activity was observed in algal tissues under Muv and Huv treatments, respectively (Fig. 1a–c). Cellular H$_2$O$_2$ concentration is the result of the balance between its production and utilization (Bowler et al., 1992). Under the serious UV-B stress, H$_2$O$_2$ concentrations increased markedly, which may be due to the weakening of degradation rather than the strengthening of production. According to the present results, CAT and POX probably had more important role in H$_2$O$_2$ detoxification than APX in C. officinalis.

TBARS is considered as a reliable indicator of lipid peroxidation in plants (Costa et al., 2002). Its concentration was stable in the Luv tissues (Fig. 2a), suggesting that C. officinalis resisted the mild stress of UV-B. A significant increase of TBARS concentration was observed in the Muv and Huv tissues at the end of exposure compared with the control (Fig. 2a), indicating that high UV-B level could result in some damage to C. officinalis.

The electrophoretic profiles of SOD isoenzymes were affected by UV-B treatment. This alteration in SOD isoenzyme profile was mainly expressed as increased intensity of some isoenzyme bands due to the exposure to UV-B (Fig. 3a). Nine SOD isoenzymes were identified, eight Mn-SOD and one Cu/Zn-SOD, which is somewhat different from previous reports in red alga Gracilaria tenuifrons, where Cu/Zn-SOD has not been observed (Rossa et al., 2002). This may be caused by species differences in SOD isofrom mechanisms.

The APX, POX, and CAT isoenzymes are also found in eukaryotic algae, which have similar enzymological and immunological properties to isoenzymes from higher plants used as biochemical indicators of resistance ability to an external stress (Takeda et al., 1998; Snao et al., 2001; Shigeoka et al., 2002; Wang et al., 2007). In the present study, POX, CAT and APX isoenzymes exhibited different patterns related to UV-B exposure. Two bands of CAT isoenzymes (CAT III and CAT IV) were eliminated under Muv and Huv treatments, while only one band, CAT IV, disappeared under Luv treatment (Fig. 3b). On the contrary, two bands of new POX isoenzymes appeared under all three doses of UV-B (Fig. 3c). Unlike POX, a new band of APX appeared only under Huv treatment (Fig. 3d). Thereby, it is speculated that the disappearing band of CAT or new bands of APX and POX isoenzyme may be closely associated with a tolerance character of C. officinalis to UV-B stress. Bechtold et al. (2008) analyzed the expression of 28 high light-responsive genes of Arabidopsis in response to environmental and physiological factors known to influence the expression of the high light-responsive gene, and found that 61% of these genes, including APX2, may be responsive to chloroplast-sourced ROS. Thus, it can be presumed that the changes in antioxidant enzyme activity on exposure to UV-B were due, at least in part, to the increase or decrease in gene expression, as the response given to other environmental factors. Moreover, macroalgae response to UV-B irradiation could be expressed at the transcriptional and post-transcriptional levels.

4 Conclusion

In conclusion, our study demonstrated that UV-B induced adaptive responses in C. officinalis, which largely depended on the dose. The activity of SOD and POX could maintain at high level when subjected to UV-B, while APX and CAT activities remained stable. Those enzymes worked together as oxygen species scavengers at low dose of UV-B. When stress picked up, especially under high dose of UV-B, the antioxidant capacity decreased. Some new bands of APX and POX were observed under high dose of UV-B, some SOD isoforms altered, and two bands of CAT disappeared on exposure to Huv treatment. Therefore, H$_2$O$_2$ and TBARS increased significantly, indicating that some oxidative damage to certain unfavorable effect in C. officinalis as a result of UV-B.

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