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Ultraviolet radiation stimulated activity of extracellular carbonic anhydrase in the marine diatom *Skeletonema costatum*

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Abstract. Previous studies have shown that reduced levels of solar UV radiation (280–400 nm) can enhance photosynthetic carbon fixation of marine phytoplankton, but the mechanisms are not known. The supply of CO_2 for photosynthesis is facilitated by extracellular (periplasmic) carbonic anhydrase (CAe) in most marine phytoplankton species. The present study showed that the CAe activity of *Skeletonema costatum* (Greville) Cleve was stimulated when treated with UV-A (320–395 nm) or UV-A + UV-B (295–320 nm) in addition to visible radiation. The presence of UV-A and UV-B enhanced the activity by 28% and 24%, respectively, at a low irradiance (PAR 161, UV-A 28, UV-B 0.9 W m^{-2}) and by 21% and 19%, respectively, at a high irradiance (PAR 328, UV-A 58, UV-B 1.9 W m^{-2}) level after exposure for 1 h. Ultraviolet radiation stimulated CAe activity contributed up to 6% of the photosynthetic carbon fixation as a result of the enhanced supply of CO_2, as revealed using the CAe inhibitor (acetazolamide). As a result, there was less inhibition of photosynthetic carbon fixation compared with the apparent quantum yield of PSII. The UV radiation stimulated CAe activity coincided with the enhanced redox activity at the plasma membrane in the presence of UV-A and/or UV-B. The present study showed that UV radiation can enhance CAe activity, which plays an important role in counteracting UV inhibition of photosynthesis.

Additional keywords: CO_2 acquisition, photoinhibition, photosynthesis.

Introduction

Photosynthetic carbon fixation by phytoplankton cells depends on the availability of CO_2 from aquatic environments. In most oceanic waters, the pH normally varies between 8.0 and 8.2, with free CO_2 (10 μM, 20°C, pH 8.2) less than 1% of the dissolved inorganic carbon (DIC; total of CO_2, HCO_3^- and CO_3^{2-}). On occasion, the pH can increase to 8.7 (Hobson *et al.* 2001) with algal blooms, leading to a further reduction in the levels of CO_2 concentration. In addition, CO_2 in seawater diffuses ~10 000-fold slower in water than in air (Raven 1999). The $K_{1/2}$ CO_2 values for Rubisco in marine diatoms have been suggested to be 30–60 μM CO_2 (Badger *et al.* 1998), ~threefold to sixfold the values recorded in ambient seawater. Most species of phytoplankton accomplish a high photosynthetic rate by operating a CO_2-concentrating mechanism (CCM) that exhibits active transport of CO_2 or HCO_3^- and supplies CO_2 to the proximity of Rubisco (Raven 1997; Raven and Beardall 2003; Giordano *et al.* 2005; Roberts *et al.* 2007). Diatom cells can acquire inorganic carbon via the direct uptake of HCO_3^- (Matsuda *et al.* 2001; Chen *et al.* 2006) or by active uptake of the ambient CO_2 (Chen and Gao 2004); thus, achieving a high photosynthetic CO_2 affinity.

Photosynthetic utilisation of HCO_3^- usually involves extracellular (periplasmic) carbonic anhydrase (CAe) as well as intracellular CAe. Carbonic anhydrase has been found in many species of phytoplankton (Giordano *et al.* 2005) and plays a key role in maintaining a sustainable photosynthetic rate at low CO_2 levels by converting HCO_3^- to CO_2 outside the plasma membrane; thus, ensuring the availability of CO_2 to the intracellular photosynthetic machinery (Sültemeyer 1998; Nimer *et al.* 1999*a*; Colman *et al.* 2002). The activity of CAe can be induced at low CO_2 concentrations (Aizawa and Miyachi 1986; Sültemeyer 1998; Bozzo and Colman 2000; Chen and Gao 2004) and regulated by changing the levels of visible radiation (Dionisio-Sese *et al.* 1990; Rawat and Moroney 1995; Sültemeyer 1998). Light of different wavelengths, such as blue light, might also affect CAe activity (Dionisio *et al.* 1989). However, it is not known whether UV radiation (280–400 nm) affects the activity of CAe.

Solar UV radiation (UVR) has been found to be detrimental to a variety of metabolic processes in phytoplankton, and can degrade certain proteins, including the enzymes involved in photosynthesis (Franklin and Neale 2002; Bouchard *et al.* 2005). However, reduced levels of solar UVR have been shown to enhance photosynthetic carbon fixation by phytoplankton assemblages with *Skeletonema costatum* (Greville) Cleve as a major component (Helbling *et al.* 2003). Recently, solar UV-A radiation has been found to drive photosynthetic carbon fixation in the absence of PAR (Gao

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et al. 2007). However, the mechanisms responsible for this enhanced photosynthesis by UVR are not known.

The marine diatom *S. costatum* is able to use both HCO_3^- and CO_2 (Korb *et al.* 1997), and can acclimate rapidly to reduced CO_2 levels by activating CAe activity, which increases the supply of CO_2 for photosynthesis (Nimer *et al.* 1998; Hobson *et al.* 2001; Chen and Gao 2003; Rost *et al.* 2006). The aim of the present study was to examine how UVR affects the CAe activity and CO_2 acquisition of this cosmopolitan diatom, which has been used as a food organism in aquaculture, but has formed harmful blooms in Chinese coastal waters in recent years (Sun *et al.* 2008).

Materials and methods

Organisms and growth conditions

Skeletonema costatum (Greville) Cleve (strain 2042) was obtained from the Institute of Oceanography, Chinese Academy of Sciences, and maintained under axenic conditions in the laboratory. The cells were cultured in filtered and sterilised seawater enriched with f/2 medium under 41 W m^{-2} (PAR, 200 μmol m^{-2} s^{-1}, 12 : 12 light : dark cycle) at 20°C, pH 8.2, with 2.0 mM total DIC. Cells at the mid-exponential growth phase were harvested when required. The harvested cells were washed once and resuspended in fresh media with the desired CO_2 level for the experiments. The DIC concentrations of the medium were adjusted by adding $NaHCO_3$ solution to CO_2-free seawater according to Gao *et al*. (1993). Low cell concentrations of ~2×10^5 cells mL^{-1} were inoculated to keep the changes in the DIC levels within 10%. The DIC was measured with a total organic carbon analyser (TOC-5000A; Shimadzu, Kyoto, Japan).The pH levels were maintained by using 20 mM barbitone [$C_8H_{12}N_2O_3$] buffer. This buffer did not affect the concentration of DIC. The dissolved gaseous CO_2 was determined on the known levels of DIC, pH, salinity and temperature according to Stumm and Morgan (1995).

Induction of CAe activity

The relationship between CAe activity and the different levels of bulk CO_2 concentrations was first established at DIC levels of 0.5, 2.0 and 4.0 mM at pH 8.7 along with the control (DIC 2.0 mM, pH 8.2) at 20°C and 41 W m^{-2} PAR. For the UV-exposed experiments, CAe activity in *S. costatum* was induced by culturing the cells (~2×10^5 cells mL^{-1}) under 2.8 μM CO_2 in the seawater (2.0 mM DIC, pH 8.7). Duplicate cultures for each treatment were run for the CAe activity analysis and two subsamples were taken from each culture for measurement.

UV radiation treatments

Following the induction of CAe, the cells were dispensed into UV-transparent quartz tubes containing the low-CO_2 (2.8 μM) medium and then exposed to simulated solar radiation either at: (i) low irradiance (W m^{-2}): PAR (395–700 nm) 161, UV-A (320–395 nm) 28 and UV-B (295–320 nm) 0.9; or (ii) high irradiance (W m^{-2}): PAR 328, UV-A 58 and UV-B 1.9. Three different radiation treatments were implemented at each irradiance level (duplicate or triplicate cultures for each treatment): (i) cells receiving UV-B, UV-A and PAR (PAB treatment, 295–700 nm) – quartz tubes covered with Ultraphan film 295 (UV Opak; Digefra, Munich, Germany); (ii) cells receiving UV-A and PAR (PA treatment, 320–700 nm) – quartz tubes covered with Folex 320 (Montagefolie No. 10155099; Folex, Dreieich, Germany); and (iii) cells receiving only PAR (P treatment, 395–700 nm) – quartz tubes covered with Ultraphan film 395 (UV Opak; Digefra). The quartz tubes were maintained in a water bath at 20 ± 1°C. The cells were harvested after 1 or 2 h of exposure for analysis of CAe activity.

A solar simulator (Sol 1200; Dr Hönle GmbH, Martinsried, Germany) equipped with a 1000-W xenon arc lamp was used for the experiment. The output irradiances of UV-B, UV-A and PAR were measured with a broadband filter radiometer (ELDONET; Real Time Computer, Möhrendorf, Germany).

Measurement of CAe activity

At the end of the exposure (1 or 2 h) to the solar radiation treatments, the cells were immediately harvested by centrifugation at 5000*g* for 10 min at 4°C, washed and resuspended (chl *a* ranged from 1.3 to 2.4 mg L^{-1}) in seawater buffered with 20 mM barbitone at pH 8.2. The cells were examined microscopically and no ruptured cells were detected. The CAe activity of the intact cells was determined by an electrometric method as described by Wilbur and Anderson (1948). A 0.2 mL cell suspension was added to 4 mL cold barbitone buffered seawater and stirred; the time required for the pH to drop from 8.2 to 7.2 was recorded after the addition of 2 mL ice-cold CO_2-saturated pure water. The temperature during the reaction was controlled at 4°C. Activity of the enzyme was calculated as follows: $EU = 10(T_0/T - 1)$, where T_0 and T are the times in seconds for the pH drop without and with the sample, respectively.

Measurements of photosynthetic CO_2 fixation and effective quantum yield

Photosynthetic carbon fixation and PSII effective quantum yield were evaluated to determine how photosynthesis responded to the radiation treatments with and without UVR. Triplicate cultures were run. The photosynthetic rate was determined using the ^{14}C method. Cells (~2×10^5 cells mL^{-1}) that had had their CAe activity induced previously (as described above) were inoculated with 0.1 mL of 0.2 μCi $NaH^{14}CO_3$ (ICN Radiochemicals, Amersham, Buckinghamshire, UK) under different radiation treatments. To test how a CAe-facilitated supply of CO_2 affects photosynthesis, incubations were carried out in the presence or absence of 200 μM acetazolamide (AZ) (Sigma, St Louis, MO, USA), which inhibits the activity of CAe, but does not penetrate the cells. After incubation for 1 h, the cells were filtered onto a Whatman GF/F filter (25 mm, Maidstone, England, UK) and then placed into 20 mL scintillation vials, exposed to HCl fumes overnight and then dried at 45°C. The assimilated ^{14}C was determined using a liquid scintillation counter (LS 6500; Beckman Coulter, Fullerton, CA, USA).

A portable pulse amplitude modulated fluorometer (PAM-WATER-ED; Walz, Effeltrich, Germany) was used to determine the chlorophyll fluorescence. Samples were taken after exposure for 1 h to the radiation treatments with or without UVR at the low and high irradiance levels to measure the effective photosynthetic quantum yield (Φ_{PSII}). The Φ_{PSII} was determined by measuring

the instant maximal fluorescence (F'_m) and the steady-state fluorescence (F_t) of light-adapted cells and calculated according to Genty *et al*. (1989) as: $\Phi_{PSII} = (F'_m - F_t)/F'_m$. In the course of measuring F'_m and F_t, the saturating pulse and actinic light were set at 4000 and 150 μmol m^{-2} s^{-1}, respectively.

Measurement of exofacial ferricyanide at the plasma membrane

To determine if the stimulated CAe activity was related to changes in the redox activity at the plasma membrane, we assessed the impacts of UVR on the redox activity by incubating the cells with ferricyanide [$K_3Fe(CN)_6$] at 500 μM, which does not penetrate intact cells and has been used as an external electron acceptor (Rubinstein and Luster 1993). Rates of exofacial ferricyanide reduction in the medium with (~2×10^5 cells mL^{-1}) or without cells were measured according to Nimer *et al*. (1998) as the change in absorbance at 420 nm. Samples from triplicate cultures were taken after 1 h exposure to PAR, PAR + UV-A or PAR + UVR at the low irradiance level. The absorbance of the supernatant after centrifugation (5000*g* for 10 min at 20°C) was measured immediately against the optical density of the control. Stock solutions of $K_3Fe(CN)_6$ were freshly prepared each time.

SDS–PAGE gel electrophoresis

We separated the protein by SDS–PAGE electrophoresis to determine whether UVR stimulates CAe protein synthesis. Low CO_2 grown cells at the exponential growth phase were exposed to the different radiation treatments with or without UVR. At the end of the exposures, the cells were harvested by centrifugation at 6000*g* for 15 min at 4°C. The concentrated cells (2×10^8 cells mL^{-1}) were treated with trypsin at a final concentration of 10 g L^{-1} at 25°C for 5 min to release the CAe and other periplasmic proteins (Nimer *et al.* 1998), and then the samples were centrifuged again at 6000*g* for 15 min at 4°C. The proteins in the supernatant were precipitated with cold acetone and kept at −20°C for 1 or 2 h, and spun for 20 min at 4°C at a speed of 12 000*g*. The retained protein pellet was dried to remove any acetone residue. For SDS–PAGE, the dried samples were dissolved in 1000 μL of buffer [0.125 M Tris–HCl (pH 6.8) and 0.15 M NaCl], and then 10 μL of the concentrated protein was loaded onto a SDS polyacrylamide gel (10% w/v). The 30-kDa CAe protein was separated in the SDS–PAGE gel and analysed using the TANON (Shanghai, China) gel imaging system (GIS3.73).

Measurement of chlorophyll a *and cell number*

Chlorophyll *a* was extracted and determined by the spectrophotometric method according to Jeffrey and Humphrey (1975). Cells were counted microscopically using a haemacytometer.

Statistical analysis

One-way ANOVAs and *t*-tests were used to examine differences among the different radiation treatments and irradiance levels. A confidence level of 95% was used in all analyses.

Results

UVR-stimulated CAe activity

The CAe activity in *S. costatum* cells increased in response to reduced CO_2 concentrations in the medium (Fig. 1). The activity was not detected under 10 μM CO_2, but developed quickly ($P < 0.01$) at CO_2 concentrations below 5.0 μM. At decreased levels of CO_2, CAe activity was fully induced in 1 h and no further increase in CAe activity was found when the induction lasted for 2 h.

When the cells with induced CAe activity were exposed to simulated solar radiation at low or high irradiance levels, the activity of CAe changed in response to both the irradiance level and the doses of light with or without UVR (Fig. 2). The CAe activity increased significantly ($P < 0.01$) when exposed to the radiation treatments with UVR (PAB) or UV-A (PA) compared with PAR (P) alone, indicating that both UV-A and UV-B enhanced the CAe activity (Fig. 2). Raising the PAR level from 41 W m^{-2} (indoor control) to the treatment levels of 175 or 356 W m^{-2} also stimulated the CAe activity ($P < 0.01$). Irrespective of the radiation treatment, 1 h exposure of the cells resulted in higher CAe activity at the low irradiance level than at the high irradiance level ($P < 0.01$). The presence of UV-A and UV-B enhanced the activity by 28% and 24%, respectively, at the lower irradiance level and by 21% and 19%, respectively, at the higher level, indicating reduced enhancement as a result of the counteracting inhibitory effects caused by UVR ($P < 0.05$). In contrast, 2 h exposures at the high irradiance level led to a significant ($P < 0.05$) reduction in the activity of CAe (Fig. 2). The activity decreased by 78% in the presence of UVR (UV-A + UV-B) compared with PAR alone ($P < 0.05$). Longer exposure (2 h) to the high irradiance level reversed the stimulation to inhibition of CAe activity.

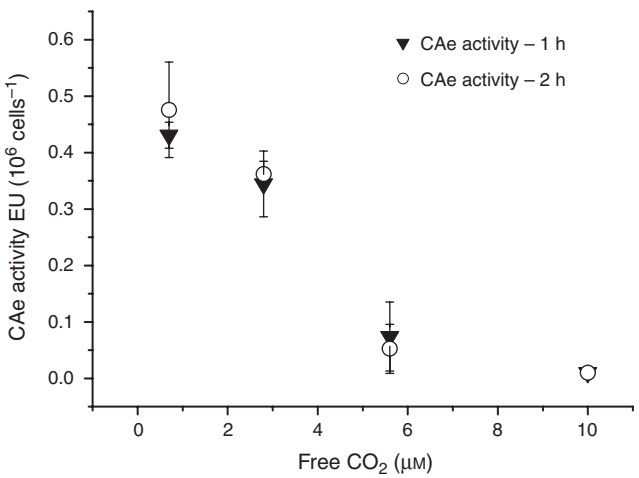

Fig. 1. Extracellular carbonic anhydrase (CAe) activity of *Skeletonema costatum* in relation to free CO_2 concentrations. Cells grown at pH 8.2, 2.0 mM total dissolved inorganic carbon (DIC), 20°C and 41 W m^{-2} PAR were re-suspended in fresh medium at DIC concentrations of 0.5, 2.0 and 4.0 mM at pH 8.7 or 2.0 mM at pH 8.2. After 1 or 2 h incubations, the cells were harvested and assayed for CAe activity. Values are mean ± s.d. ($n = 4$).

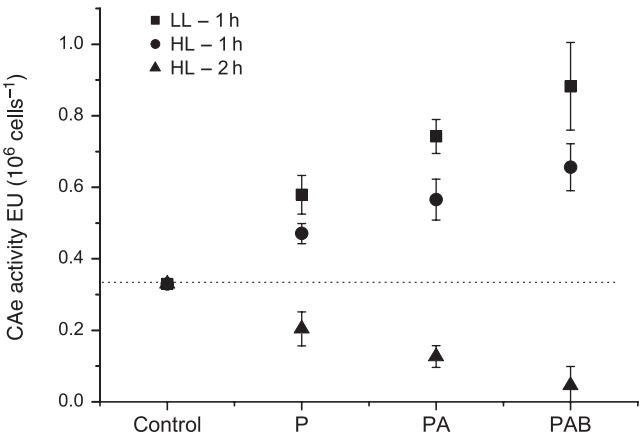

Fig. 2. Extracellular carbonic anhydrase (CAe) activity of *Skeletonema costatum* in relation to different levels of PAR and UV radiation (UVR). Cells were pre-incubated at pH 8.7, 2.0 mM total dissolved inorganic carbon (2.8 μM CO_2) and 41 W m^{-2} PAR for 1 h and then exposed to PAR (P), PAR + UV-A (PA) and PAR + UV-A + UV-B (PAB) treatments at low (LL: PAR 175, UV-A 40 and UV-B 1.3 W m^{-2}) or high (HL: PAR 356, UV-A 82 and UV-B 2.6 W m^{-2}) irradiance levels for 1 or 2 h in parallel with the control at 41 W m^{-2} PAR. Values are mean ± s.d. ($n = 4$).

Photosynthetic carbon fixation and quantum yield

As the CAe activity of the low CO_2 grown cells was stimulated by UVR, photosynthetic carbon fixation was investigated to determine whether the rate was raised by enhanced CO_2 supply facilitated by the stimulated CAe activity. The membrane-impermeable inhibitor of CAe, AZ, was used when measuring the photosynthetic CO_2 fixation under different radiation treatments. UVR significantly ($P < 0.01$) inhibited the photosynthetic CO_2 fixation compared with PAR alone, regardless of the AZ. A UV-A level of 28 W m^{-2} (100 kJ m^{-2}) and a UV-B level of 0.9 W m^{-2} (3.2 kJ m^{-2}) inhibited the photosynthetic rates by 16% and 13% under the low irradiance level, respectively (Fig. 3*A*). Photosynthetic CO_2 fixation decreased significantly ($P < 0.01$) in the presence of AZ compared with that recorded in the absence of AZ, indicating that inhibited CAe resulted in reduced photosynthetic rates (Fig. 3*A*). UVR-induced photosynthetic inhibition was 29% and 35% in the cells treated without or with AZ ($P < 0.05$), respectively. This unequal inhibition of photosynthetic carbon fixation caused by UVR could be attributed to UVR-stimulated CAe activity and the subsequently stimulated CO_2 supply, which offset part of the UVR-inhibition of CO_2-limited photosynthesis.

The effective quantum yield (Φ_{PSII}) was 0.61 ± 0.02 for *S. costatum* cells cultured under PAR of 41 W m^{-2}. When these cells were exposed to the simulated solar radiations, Φ_{PSII} declined with UVR or elevated levels of PAR (Fig. 3*B*). UV-A and UV-B inhibited Φ_{PSII} by 22% and 23%, respectively. When the photosynthetic carbon fixation and effective quantum yield were compared, the former was decreased less by UVR than the latter; their ratio also showed higher values under the radiation treatments with UVR (Fig. 3*C*). Again, these higher ratios coincided with the higher CAe activity in the presence of UVR.

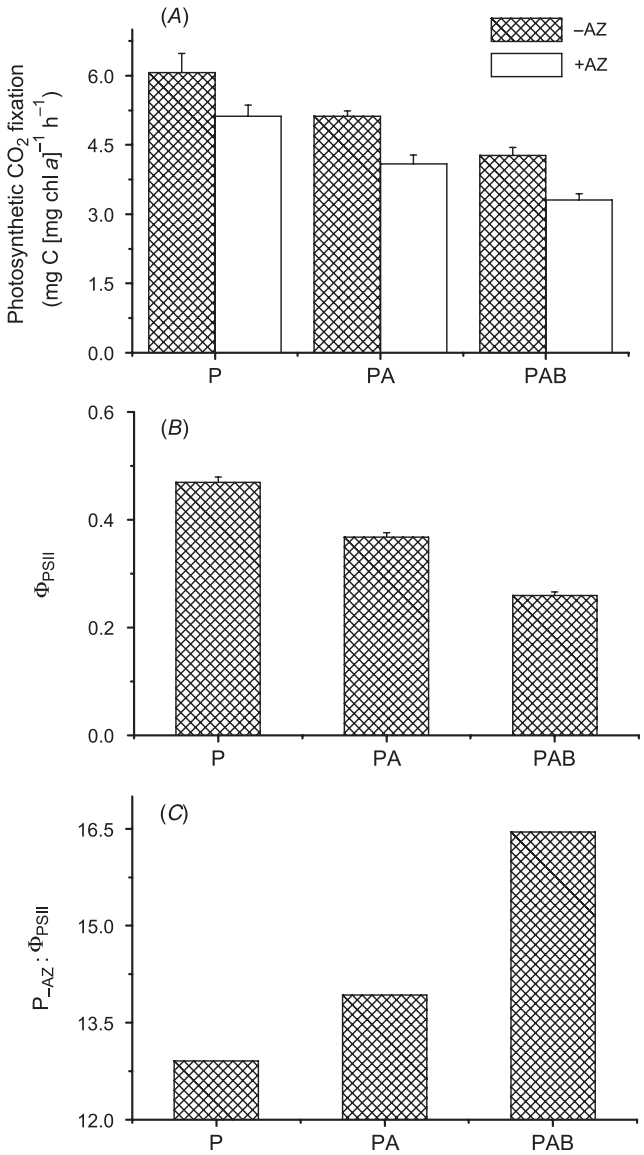

Fig. 3. (*A*) Photosynthetic CO_2 fixation in the absence (P_{-AZ}) or presence (P_{+AZ}) of acetazolamide (AZ), (*B*) effective photosynthetic quantum yield (Φ_{PSII}) and (*C*) the ratio of P_{-AZ} to Φ_{PSII} for *Skeletonema costatum* cells under different radiation treatments (see Fig. 2). Cells were pre-incubated at pH 8.7, 2.0 mM total dissolved inorganic carbon and 41 W m^{-2} PAR for 1 h and then exposed to PAR (P), PAR + UV-A (PA) and PAR + UV-A + UV-B (PAB) treatments for 1 h at low irradiance levels. ^{14}C assimilation was estimated in the presence or absence of 200 μM AZ. The initial value of Φ_{PSII} was 0.61 ± 0.02. Values are mean ± s.d. ($n = 3$).

Plasma membrane redox activity in relation to UV radiation

In the presence of UVR, the reduction of ferricyanide occurred at faster rates (Table 1). Compared with the cultures under PAR alone, cultures exposed to PAR + UV-A had 4% higher reduction rates, and the addition of UV-B enhanced the reduction by another 2% ($P < 0.01$). The plasma membrane redox activity appeared to increase with both UV-A and UV-B.

Table 1. The reduction of ferricyanide (A_{420}nm) with intact cells of *Skeletonema costatum* under different radiation treatments

Cells were pre-incubated at pH 8.7, 2.0 mM total dissolved inorganic carbon and 41 W m^{-2} PAR for 1 h and then exposed to PAR (P), PAR + UV-A (PA) and PAR + UV-A + UV-B (PAB) treatments for 1 h at a low irradiance level. The reduction of ferricyanide (500 μmol L^{-1}) in samples without cells was set as the control. Values are mean ± s.d. (n = 3)

Radiation treatments	Ferricyanide (μmol L^{-1})	
	–cells	+cells
P	500.0 ± 0.6	480.0 ± 2.6
PA	500.0 ± 1.0	459.0 ± 3.0
PAB	499.3 ± 0.6	449.3 ± 1.2

CAe protein

SDS–PAGE electrophoresis showed that the CAe protein appeared to increase in the presence of either UVA or UVB compared with PAR alone for cells exposed to low or high irradiation levels for 1 h, but decreased after 2 h exposure to the high irradiance level (Fig. 4). The estimated amount of CAe, either on a basis of per cell or normalised to the amount of total periplasmic proteins, was higher in the presence of UVR by up to 28% compared with the PAR alone treatment (Fig. 5). In addition, the periplasmic proteins increased by up to 11% in the cells exposed to PAR + UVR compared with those exposed to PAR alone (Fig. 5).

Discussion

The CAe activity of *S. costatum* can be induced rapidly (within minutes) in response to inorganic carbon limitation under visible radiation (Nimer *et al.* 1998). In the present study, the fully induced CAe activity under PAR of 41 W m^{-2} was further stimulated when the cells were exposed to moderate levels of solar UV doses, 104 kJ m^{-2} (low irradiance level for 1 h) and 216 kJ m^{-2} (high irradiance for 1 h). However, a higher UV dose of 432 kJ m^{-2} (high irradiance for 2 h) reduced the CAe activity. UVR-stimulated CAe activity contributed ~6% of the photosynthetic carbon fixation resulting from the enhanced supply of CO_2 as revealed by the CAe inhibitor AZ (Fig. 3*A*). The present study also showed that, at the low level of solar irradiance, UVR induced less inhibition of carbon fixation than Φ_{PSII}, and this unbalanced inhibition was coupled with UVR-stimulated activity of CAe (Fig. 6). Enhanced facilitation of the conversion of HCO_3^- to CO_2 at the plasma membrane by the UVR-stimulated CAe must have added to the CO_2 supply, and played an important role in counteracting UV inhibition of photosynthesis. In addition, it was recently found that reduced or frequently fluctuating levels of solar UV radiation enhanced photosynthetic carbon fixation by a phytoplankton assemblage by up to 7–30% (Barbieri *et al.* 2002; Helbling *et al.* 2003; Gao *et al.* 2007). UVR-stimulated CAe activity, facilitating the dehydration of HCO_3^- to CO_2, could be partially responsible for the enhanced photosynthetic carbon fixation, particularly under the CO_2-limited conditions often found during algal blooms. Nevertheless, at the high light level, UVR caused a reduction in CAe activity and resulted in much lower effective quantum yield (Fig. 6). High levels of radiation could lead to degradation of the CAe protein (Figs 4, 5), as found for Rubisco and D1 protein (Bischof *et al.* 2002; Bouchard *et al.* 2005), thus, contributing to the greater photoinhibition of photosynthesis.

The CAe activity of *S. costatum* has been shown to be associated with the redox activity in the plasma membrane under visible light (Nimer *et al.* 1998, 1999*b*). In the present study, UVR + PAR caused higher rates of redox activity than PAR alone (Table 1), coinciding with stimulated CAe activity by UVR (Fig. 2); thus, showing that UVR-stimulated CAe activity in

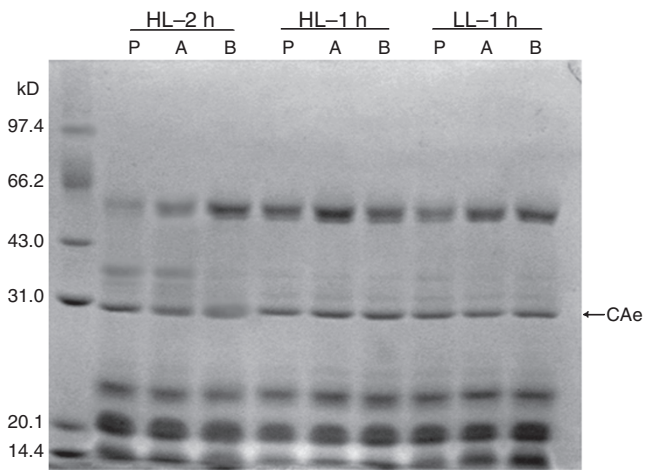

Fig. 4. SDS–PAGE analysis of the periplasmic proteins obtained from the *Skeletonema costatum* cells after trypsin treatment. The low CO_2 grown cells were exposed to PAR (P), PAR + UVA (PA) and PAR + UVA + UVB (PAB) treatments for 1 h or 2 h at the high irradiance level (HL) or for 1 h at the low (LL) irradiance level. Cells were harvested to a concentration of 2×10^8 cells mL^{-1} and treated with trypsin. Concentrated soluble proteins (10 μL) were loaded onto each track. The proteins were stained with Coomassie Brilliant Blue G. The carbonic anhydrase (CAe) protein (30 kD) under different solar radiation treatments is shown in the figure.

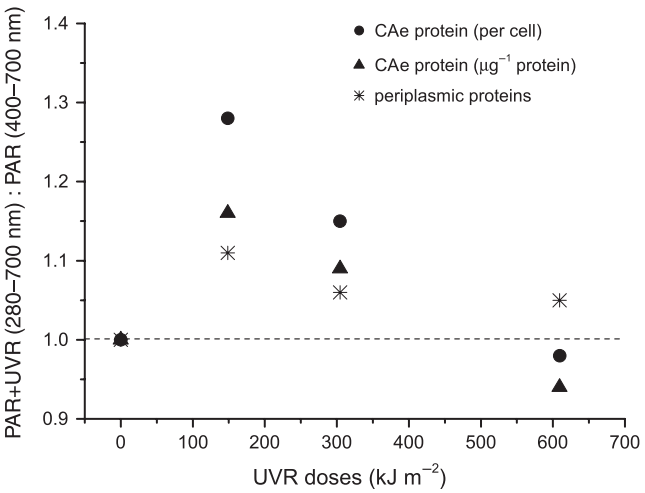

Fig. 5. Ultraviolet radiation dose-dependant carbonic anhydrase (CAe) protein and total periplasmic proteins in contrast to that under the PAR treatment. The results are based on the relative amounts estimated from the data in Fig. 4 with a TANON gel imaging system (GIS3.73) and expressed on a basis of per cell or normalised to the amount of total periplasmic proteins in the electrophoresis.

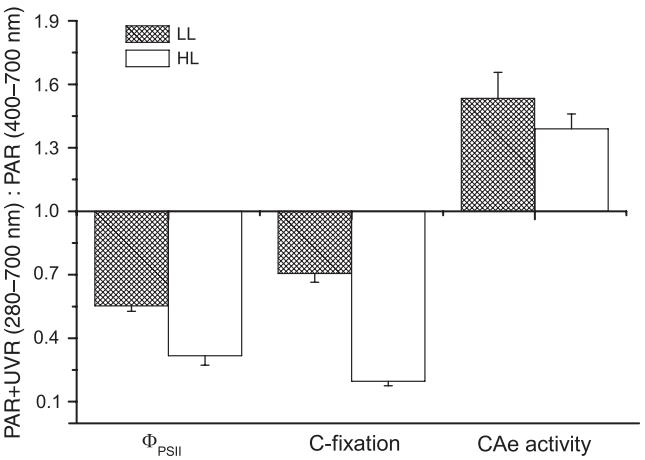

Fig. 6. Ultraviolet radiation (UVR) induced effects indicated as the ratio of PAR + UVR to PAR treatments on the effective quantum yield of PSII (Φ_{PSII}), photosynthetic carbon fixation (C-fixation), carbonic anhydrase (CAe) activity in the low CO_2 grown cells of *Skeletonema costatum* under low (LL) or high (HL) irradiance levels. Values are the mean ± s.d. (n = 3–4).

S. costatum was related to an enhanced plasma redox process. UVR-triggered signalling might have been involved in the activation of the CAe in *S. costatum* because it is known that plasma membrane redox activity is associated with the UV-B and UV-A signal transduction pathway in higher plants (Long and Jenkins 1998). In addition, the presence of UVR in the present study appeared to increase the amount of CAe protein (Figs 4, 5).

A study with *Dunaliella tertiolecta* Butcher showed that UV-B inhibited carbon fixation, but increased significantly the intracellular DIC pools (Beardall *et al.* 2002). Although UVR-sensitive CO_2 or HCO_3^- acquisition mechanisms were not explored by these authors, it is likely that the enhancement of CAe activity might have been involved in facilitating CO_2 transport across the periplasmic space, leading to increased intracellular DIC pools. Although UV-B can affect cell membrane permeability (Sobrino *et al*. 2004) and counteract the accumulation of intracellular inorganic carbon, such an effect appeared to be significant only under CO_2-enriched and low-pH conditions (Sobrino *et al*. 2008). In the present study, CAe was induced under CO_2-limited conditions; therefore, exposure to UVR up to the moderate levels is unlikely to lead to depolarised membrane of *S. costatum*.

In nature, *S. costatum* cells are usually circulated up and down in the ocean as a result of mixing or waves, and experience frequently reduced levels of fluctuating solar UVR. The data from the present study indicate that the acquisition of CO_2 in *S. costatum* can be achieved faster with moderate levels of UVR because of its enhancing effect on the activity of CAe. Further studies need to be conducted to understand the potential mechanism of the stimulating effects of UVR.

Acknowledgements

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