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Effects of temperature, pH, and UV radiation on alkaline phosphatase activity in the terrestrial cyanobacterium *Nostoc flagelliforme*

Ping Li · Wenhua Liu · Kunshan Gao

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Abstract Cyanobacteria produce phosphatases in response to phosphorus deficiency as some other autotrophs. However, little has been documented on the effects of key climate change factors, such as temperature rise and solar UV radiation (280–400 nm), on cyanobacterial alkaline phosphatase activity. Here, we found that the terrestrial cyanobacterium *Nostoc flagelliforme* showed higher activity of the enzyme with increasing temperature and pH levels, exhibiting maximal values at 45 °C and pH 11, respectively. However, when exposed to solar radiation in the presence of UV-A (320–400 nm) and UV-B (280–320 nm), significant reduction of the enzyme activity was observed at a photosynthetically active radiation (PAR) level of 300 $W m^{-2}$ (1,450 μmol photons m^{-2} s^{-1}), which is equivalent or lower than the noontime level of solar PAR at the organism's habitats. UV-A and UV-A + UV-B induced about 21 and 39 % inhibition of the enzyme activity in the 3-h exposures. The decrease in the activity of phosphatase can be attributed to the UV radiation-induced inactivation of the enzyme and indirectly to the UV radiation-induced production of reactive oxygen species.

Keywords Alkaline phosphatase · *Nostoc flagelliforme* · pH · Temperature · UVR

P. Li · W. Liu
Marine Biology Institute, Shantou University,
Daxue Road No.243,
Shantou, Guangdong 515063, China

K. Gao (✉)
State Key Laboratory of Marine Environmental Science,
Xiamen University,
Xiamen, Fujian 361005, China
e-mail: ksgao@xmu.edu.cn

Introduction

Nostoc flagelliforme (Berk & Curtis) Bornet and Flahault is a terrestrial, macroscopic, filamentous cyanobacterium which is distributed in arid and semiarid areas, including Algeria, China, Czechoslovakia, France, Mexico, Mongolia, Morocco, Russia, Somalia, and USA (Gao 1998). It is one of the most important edible cyanobacteria species in China, having been regarded as a food delicacy for more than 2,000 years (Gao 1998). Biologically active compounds isolated from *N. flagelliforme* also showed antiviral activities (Kanekiyo et al. 2007; Jia et al. 2007).

The habitats of *N. flagelliforme* are characterized by conditions such as intense solar radiation, extreme temperature differences, and extreme desiccation (Qian et al. 1989; Gao 1998; Liu et al. 2010b). Limited information is available on the nutrient uptake of this alga, and rehydration plays an important role in its nutrient uptake, growth, reproduction, survival, and yield when it is exposed to long periods of drought (Diao 1996; Qiu et al. 2004a, b; Gao and Zou 2001; Gao and Ye 2007). Cell wall enzymes such as alkaline phosphatase (APase), which plays an important role in phosphorus uptake, may be activated during rehydration. Similar to several other photosynthetic autotrophs, cyanobacteria produce phosphatases in response to phosphorus deficiency in their external environment (Hernández et al. 2003; Whitton et al. 2005). To date, several phosphatases have been identified in different cyanobacterial species (Bhaya et al. 2002). On activation, phosphatases external to the cytoplasmic membranes can catalyze the release of orthophosphate from organic phosphorus compounds and thereby increase the amount of available phosphate in the immediate environment (Chróst and Siuda 2002; Whitton et al. 2005). Environmental factors, such as pH, temperature, and light (including ultraviolet radiation

[UVR], 280–400 nm), may affect the activity of *N. flagelliforme* phosphatase during rehydration. In fact, temperature changes affect the stability of the enzyme, velocity of the breakdown of the organic phosphate, and enzyme–substrate affinity (Hernández et al. 2003). The optimum temperature for the alkaline phosphatase activity (APA) of marine seaweeds ranges from 25 to 30 °C (Hernández 1996). The optimal conditions for the activity of the alkaline phosphatase of *Nostoc commune* UTEX 584 were determined to be a pH of 7.6 and a temperature of 32 °C. Chróst and Siuda (2002) found that APases react optimally in the pH range of 7.6 to 9.6. Hoppe (2003) reported that the optimum pH for acidic and alkaline phosphatases was ph 4 to 6 and ph 8.3 to 9.5, respectively. However, to the best of our knowledge, no research has been conducted on the APA of *N. flagelliforme*.

The activity of an external enzyme is not expected to be closely associated with exposure to photosynthetically active radiation (PAR), at least in the short term. However, comparisons of light and dark conditions showed both positive (e.g., *Gelidium sesquipedale*) and negative (e.g., *Corallina elongata*) effects on the activity of red algae phosphatase (Hernández 1996). Antarctic samples of dry desert rocks containing *Chroococcidiopsis* and the mosses *Fontinalis squamosa*, *Rhynchostegium riparioides*, and *Warnstorfia fluitans* showed lower alkaline phosphatase activity in the light than in the dark (Whitton et al. 2005). In nature, *N. flagelliforme* is exposed to high solar radiation and is thus susceptible to UVR (Gao 1998; Gao and Ye 2007). This is particularly important in view of the increase in solar UV-B radiation (280–320 nm) because of the reduction in the amount of stratospheric ozone due to atmospheric pollutants (Pieter 2007). UVR, particularly UV-A (320–400 nm), has been reported to exert certain positive effects, such as an enhancement of carbon fixation in phytoplankton by UV-A (Gao et al. 2007) and facilitation of DNA damage repair by UV-A (Sinha and Häder 2002). Moreover, UVR, especially UV-B (280–320 nm), has also been shown to have a significant influence on a range of algal biochemical processes, including nutrient uptake, pigmentation, photosynthesis, growth, and biochemical composition (Vass et al. 2000; Xiong 2001; He and Häder 2002; Häder et al. 2007; Fiorda Giordanino et al. 2011). However, to date, the effect of UVR on APA remains largely unstudied, and the impact and magnitude of UVR-induced changes, if any, on APase function are poorly understood. Laboratory studies have, however, shown decreased APA of the phytoplankton community on exposure to UVR (Wetzel et al. 1995; Tank and Xenopoulos 2005).

In the natural environment where *N. flagelliforme* grows, pH surrounding its colonies or single filament, when there is enough moisture, increases with an increase of solar radiation due to photosynthetic carbon fixation; therefore, the organism may experience changes in both pH and solar UVR during daytime. Therefore, the aim of this study was to examine how pH and UVR affect the APase activity in *N. flagelliforme*, which is the key enzyme for the organism to survive in arid areas.

Materials and methods

Experimental organism and culture conditions

Samples of *N. flagelliforme* were collected from Sunitezuoqi (43.85° N, 113.7° E) of Inner Mongolia and stored dry for 3 years until they were used for experiments. The water content of these dried field samples was approximately 0.1 g g^{-1} dry weight (DW). Before the samples were used for the assays, the dried field samples were rinsed three times with distilled water and rehydrated at 60 μmol photons m^{-2} s^{-1} and 25 °C in phosphate-free BG_{11} medium for 24 h.

Phosphatase assay

N. flagelliforme filaments were randomly selected and cut into 2–3-cm-long pieces. Samples of 23–30 mg dry weight were used for each assay. APA (phosphomonoesterase) was assayed using the colorimetric method with para-nitrophenyl phosphate (*p*-NPP; Sigma, USA) as described by Turner et al. (2001). Samples were added to 25 mL of phosphate-free BG_{11} medium in a 50-mL conical flask and shaken for 5 min under the conditions chosen for the subsequent tests. Assays were performed using 0.1 mL of the substrate to achieve a final concentration of 100 μM. The flasks were incubated at 25 °C (except for the temperature experiment) in a shaker, which was running at a speed of 70–80 strokes min^{-1}, under a light level of 50–60 μmol photons m^{-2} s^{-1}. At the end of the assay, approximately 8 mL of the medium was collected using a 10-mL syringe and passed through a 25-mm Whatman GF/F filter paper. Absorbance was then measured at 410 nm immediately after filtration using a spectrophotometer (Shimadzu UV-2501PC; Shimadzu Corporation, Japan). Samples with values of >0.8 were diluted with the culture medium. The activity was determined using calibration curves constructed from para-nitrophenol (pNP; Sigma, USA) standards (0–50 μM) in the assay medium. Enzyme activity was expressed as micromole pNP released per gram dry weight per hour. Control (no substrate) and blank vials (substrate and buffer only) were used for each measurement. The values of the blank and control vials were subtracted from the final measured values. Only a small amount of chemical hydrolysis was detected from the substrates. Four replicates were employed for all the analysis. This assay method permits the incubation of the whole filaments, so it is an estimate the overall enzymatic activity.

Examination of pH and temperature effects

Before the samples were used for the effect of pH and temperature on APA, the dried field samples were rehydrated under the condition mention above. The phosphatase activity was assayed at a range of pH values. The phosphate-free BG_{11} medium was buffered to different pH values by adding 3,3-dimethyl-glutaric acid for pH5.0–6.0, *N*-(2 hydroxyethyl) piperazine–*N′*-(2 ethanesulfonic acid) HEPES for pH7.0–8.0, or glycine for pH9.0–12.0, with final concentrations of 20 μM. Each pH level was strictly checked using a Mettler Toledo SevenEasy S20K pH electrode, which was calibrated with standard NBS buffer. The samples were soaked in the test pH level for 30 min before the APA measurement. APA activities of algal samples under these pH levels were then assayed as mentioned above.

The temperature-dependent phosphatase activity of *N. flagelliforme* was also examined. The temperature was adjusted and maintained by using illuminating incubators (LRH-250-G; Zhujiang Instruments Ltd., China). Phosphatase activity was measured at nine different temperatures (5, 10, 15, 20, 25, 30, 35, 40, and 45 °C). The samples were soaked in the test temperature level for 30 min before the APA measurement. And the assays were performed at pH8.0.

Kinetic parameters

The kinetic properties of APase were characterized using a final *p*-NPP concentration in the range of 5–500 μM. The kinetic assays were performed at pH8.0 and 25 °C. The velocity of enzyme catalysis increases with an increase in the substrate concentration up to a certain point that approaches a maximum velocity (V_{max}). The Michaelis constant (K_m) is the substrate concentration at which the velocity is half the maximum velocity. K_m is an indication of the affinity of the enzyme for the substrate: the lower the K_m, the higher is the affinity. These parameters are described by the Michaelis–Menten equation:

$$V = V_{max} \times S/(K_m + S) \quad (1)$$

where V is the velocity of the reaction at a given time point and S is the substrate concentration. The kinetic parameters (V_{max} and K_m) were calculated using a Lineweaver–Burk plot ($1/V$ vs. $1/S$, where the y intercept is $1/V_{max}$ and the gradient is K_m/V_{max}).

UV radiation measurements and treatments

Experiments were performed using a solar simulator (Sol 1200W; Dr. Hönle, Martinsried, Germany). The output of the irradiance of the simulator was measured using a broadband ELDONET filter radiometer (Real Time Computer Inc., Germany), which has three channels: the first one measures PAR (400–700 nm); the second one measures UV-A (315–400 nm); and the third one measures UV-B (280–315 nm). This device has been universally recognized (certificate no. 2006/BB14/1) and is calibrated regularly. Before the samples were used for the UV exposure, the dried field samples were rinsed three times with distilled water and rehydrated at 60 μmol photons m^{-2} s^{-1} and 25 °C in phosphate-free BG_{11} medium for 24 h. The samples were placed on the glass petri dish (inner diameter, 92 mm; depth, 14 mm) without cover, which was then introduced into a water bath, and the water was circulated with a refrigerating circulator to maintain the water temperature at 25 °C. Before exposure, the filaments in the dish were separated and ensured not to shade each other. The outer surface of the dishes was painted black to avoid reflectance during exposure to the lamp. The dishes with the samples were exposed to the following treatments for 3 h: (1) for PAR + UV-A + UV-B (PAB), the dishes were covered with 295-nm cut-off filters (Ultraphan; Digefra, Germany), transmitting irradiances above 295 nm; (2) for PAR + UV-A (PA), the dishes were covered with 320-nm cut-off filters (Montagefolie; Folex, Germany), transmitting irradiances of above 320 nm; and (3) for PAR, the dishes were covered with 395-nm cut-off filters (Ultraphan UV Opak; Digefra, Germany). The transmission spectra of these filters have been previously reported (Zheng and Gao 2009). The intensity of PAR, UV-A, and UV-B radiation applied in this study was 300.0 Wm^{-2}, 66.25 Wm^{-2}, and 2.29 Wm^{-2}, respectively, which is near the normal noon irradiance levels in Inner Mongolia (Bai and Wang 2004). The biologically weighted UV-B irradiance (calculated according to the method described by Setlow (1974), normalized at 300 nm) was 0.22 Wm^{-2}. Alkaline phosphatase activities of algal samples after irradiance for 1 h, 2 h, and 3 h were then assayed as mentioned above.

Determination of photochemical efficiency

The photochemical efficiency of PSII (Φ_{PSII}) was measured using a pulse–amplitude-modulated fluorometer (PAM-control WATER-ED, Walz, Germany) according to Genty et al. (1989) as follows:

$$\Phi_{PSII} = \Delta F \Big/ F'_m = \left(F'_m - F_t\right) \Big/ F'_m, \quad (2)$$

where F'_m represents the instantaneous maximum fluorescence and F_t the steady-state fluorescence of light-adapted samples. The saturating light pulse was 5,200 μmol photons m^{-2} s^{-1} with 0.8 s duration. Light at measurement was approximately 0.3 μmol photons m^{-2} s^{-1}, and the actinic irradiance was 60 μmol photons m^{-2} s^{-1}.

Statistical analysis

One-way analysis of variance followed by post hoc Tukey's test was used to determine the statistical significance of differences. A confidence level of 95 % was used in all analyses.

Results

The APA recovered to 11.63 and 11.72 μmol pNP g^{-1} DW h^{-1} under the dark and light conditions in 30 min, respectively (Fig. 1). Although slight increases in APA occurred with the extension of rehydration time, no significant change over time was noted even after rehydration for 24 h (Fig. 1). Furthermore, no significant differences were found between the recovery of APA under dark and light conditions. This implied that light had no effect on the recovery of APA.

The APA in *N. flagelliforme* was very low at pH values of 5 and 6 (Fig. 2a). The activity was 3.70 μmol pNP g^{-1} DW h^{-1} at pH7, and it increased dramatically with an increase in pH, up to pH level of 11. The APA was 11.04, 14.37, 16.99, and 19.75 at pH values of 8, 9, 10, and 11, respectively. A marked decrease in APA was found at pH12 (Fig. 2a). The optimal pH and temperature for APA in *N. flagelliforme* were 11.and 40 °C, respectively. The APA gradually increases with temperature from 5 to 40 °C (Fig.2b). Only a slight decrease of APA was found at the extremely high temperature (45 °C), indicating that it has a high resistance to high temperature which usually occurs in their habitats.

The Michaelis–Menten kinetics of APA in *N. flagelliforme* is presented in Fig. 3. The K_m and V_{max} values were 59.20 μM and 19.82 μmol pNP g^{-1} DW h^{-1}, respectively. The K_m of *N. flagelliforme* is quite low as compare to others organisms, e.g., K_m of *Palustriella commutata* var. *falcata* is 391 μM (Turner et al. 2003), and K_m of *Nostoc calcicola* is 1,200 μM (Verma et al. 1993). The relatively low K_m value for alkaline phosphatase (59.20 μM) indicated a high affinity of alkaline phosphatase for the substrate, and this may have important implications for phosphorus uptake.

We exposed the filaments to three radiation treatments using a solar simulator to investigate the effect of high-light conditions and solar UVR on APase, as well as the photochemical efficiency. When *N. flagelliforme* was exposed for 1 h under the three radiations,

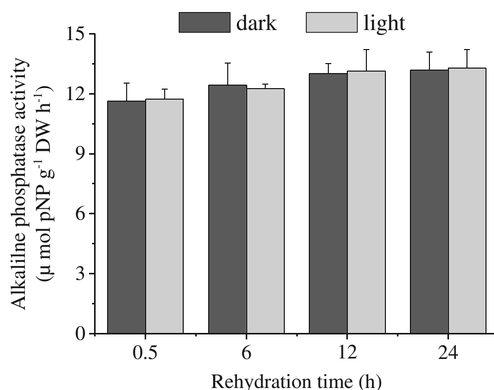

Fig. 1 APA of *N. flagelliforme* with rehydration time under dark and light conditions. The vertical bars indicate standard deviations (*n*=4)

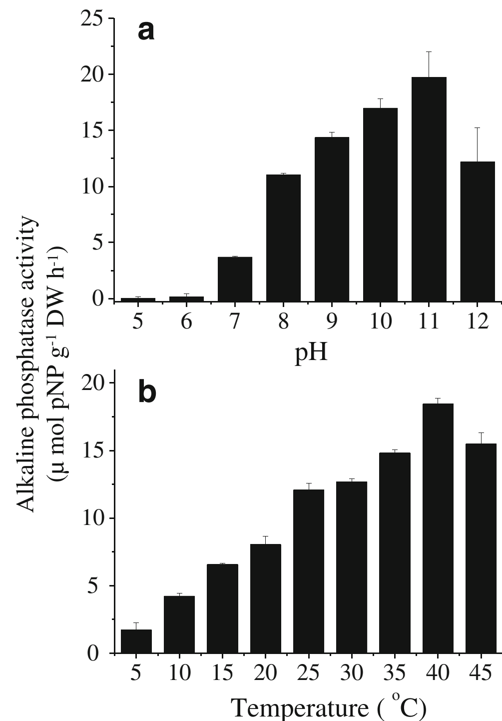

Fig. 2 The effect of pH (**a**) and temperature (**b**) on the APA of *N. flagelliforme*. The samples were rehydrated for 24 h and followed by soaking in the test pH or temperature level for 30 min before the APA measurement. The *vertical bars* indicate standard deviations (*n*=4)

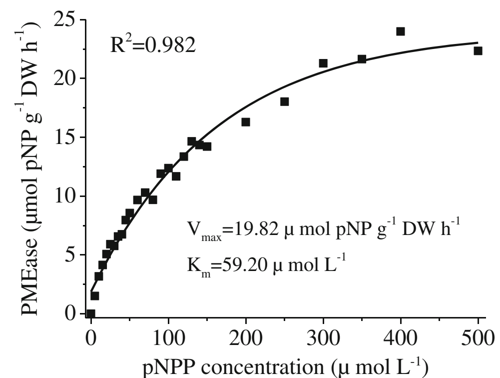

Fig. 3 Michaelis–Menten plots of the APA of *N. flagelliforme*, which were performed at pH8.0 using a final *p*-NPP concentration in the range of 5 to 500 μM. The K_m and V_{max} values were 59.20 μM and 19.82 μmol pNP g^{-1} DW h^{-1}, respectively

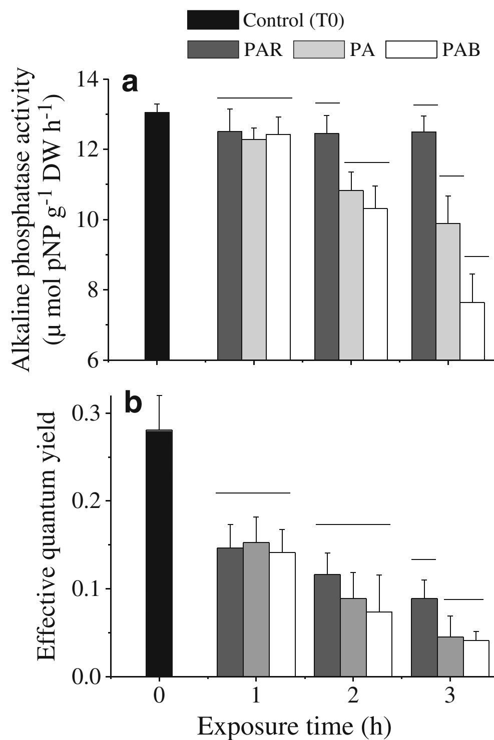

Fig. 4 Effect of ultraviolet radiation on the APA of *N. flagelliforme*. The radiation was supplied by a solar simulator and the PAR, UV-A, UV-B, and biologically effective UV-B irradiance (weighted with Setlow's DNA action spectrum) were 300.0, 66.25, 2.29, and 0.22 W m^{-2}, respectively. The *horizontal lines* represent significant differences between radiation treatments and the *vertical bars* indicate standard deviations (n=4)

protocols did not result in any significant difference from the alkaline phosphatase activity. However, 2 h of exposure to PA and PAB conditions significantly decreased the alkaline phosphatase activity by 11.9 and 19.5 % of that in PAR, respectively (Fig. 4a). The alkaline phosphatase activity decreased with time—activities of 82.9 and 61.5 % of the PAR were found when subjected to irradiance with PA and PAB for 3 h, respectively. In other words, UV-A significantly inhibited the activity of APase, and UV-B significantly enhanced this inhibition (Fig. 4a). Compared to APA, the effective quantum yield decreased much faster on exposure to a solar simulator. One hour exposure decreased the yield from 0.28 to 1.46±0.027, 1.52±0.029, and 1.41±0.026 for PAR, PA, and PAB, respectively (Fig. 4b). The yield continued to decrease with increase in exposure time. Further, UV-A or UV-A + UV-B augmented the decrease, but no significant difference was noted between the values after a 1-h exposure and those after a 2-h exposure. However, UV exposure (PA and PAB treatments) significantly decreased the effective quantum yield of *N. flagelliforme* when the filaments were exposed for 3 h (Fig. 4b).

Discussion

Evidence shows that alkaline phosphatase activity can persist for long periods even in the absence of organisms' biological activity. For instance, particle-free samples of water from the Red Sea retained 50 % of the initial APase (PMEase) activity, even after 3 to 6 weeks (Li et al. 1998). Preserved algal samples sometimes retain obvious APase (PMEase) activity even after many months of storage, whereas dried samples of cyanobacteria, such as *N. commune* and *Calothrix parietina*, retain much of their original activity when examined 2 years later (Whitton et al. 2005). Cyanobacteria growing abundantly on limestone rock samples obtained from the Aldabra Atoll in the Indian Ocean showed marked activity even 25 years after being sampled (Whitton et al. 2005). *N. flagelliforme* is one of the most environmentally resistant species in arid areas (Qian et al. 1989; Gao 1998). Therefore, it is not surprising to note that APases of *N. flagelliforme* samples stored dry for more than 3 years recovered to a very high level soon after rehydration (Fig. 1). The ability to retain enzyme activity for long periods and to rapidly recover the activity on rehydration may be important for their survival. Since most of the lifetime of *N. flagelliforme* was hair-like and no physiological activity (Qian et al. 1989; Diao 1996; Qiu et al. 2004a, b), on rehydration, it must recover enzyme activity to catalyze the organic P compounds and release orthophosphate before it can be absorbed. In addition, it is well known that increased phosphatase activity is an indicator of limited availability of phosphorus. The presence of high phosphatase activity indicted that the presence of organic phosphorus within the filaments or in the external environment is very important for the growth of *N. flagelliforme*.

Under natural conditions, after rain or the accumulation of morning dew, cyanobacterial mats are immersed in water. Such an aqueous film can be alkaline because this alga grows in strongly alkaline soils (pH 8.0–9.5) (Hu et al. 1987; Diao 1996) and the pH of nature rain can be alkaline (Liu et al. 2010a). The alkalinity may be beneficial for APA. The optimal pH for APA appears to be approximately ph 11, which is considerably higher than the environmental pH (Fig. 2a), and also higher than the pH required by other species (Chróst and Siuda 2002; Hoppe 2003). However, several studies have found that the optimum pH for APA was much higher than the typical pH of their environment (Grainger et al. 1989; Hernández et al. 2003; Štrojsová et al. 2003). For example, Grainger et al. (1989) found the cyanobacterium *Calothrix viguieri* isolated from a mangrove root had an optimal pH of 12.2 for APA. The exact reasons for this high optimal pH remain unknown. It might, however, be attributed to the high buffer concentration used in the assay and the high substrate concentration applied (Štrojsová et al. 2003). Temperature is a key factor in the

control of physiological activity in living organisms, including cyanobacteria. Although extremely high temperatures (>45 °C) may affect the stability of the enzyme and hamper the progression of its catalytic activity, increased temperature (from 5 to 40 °C) will enhance the catalyzation of organic phosphate, as shown in Fig. 2b. The presence of enzymes resistant to high temperatures is important for the survival of this organism because *N. flagelliforme* experiences extreme temperature changes in its natural habitat. Although the annual mean temperature in its habitats is approximately 2.2–8.6 °C, the highest temperature in summer can reach 35 °C, and the extreme high temperature in the surface of arid bare lands can rise to approximately 66 °C in summer (Qian et al. 1989).

The observed phosphatase kinetics of *N. flagelliforme* resembles those of pure enzyme systems (Fig. 3) and display a relatively low K_m value for AP (59.20 μM), as compared to that observed in other organisms (Fig. 3). For example, the moss *P. commutata* var. *falcata* displays a high K_m value of 391 μM (Turner et al. 2003), while another cyanobacterium *N. calcicola* shows a K_m value of 1,200 μM. The high affinity of alkaline phosphatase for the substrate may play an important role in the growth of *N. flagelliforme* because the substrate concentrations in its environment are typically low (Qian et al. 1989).

It is well known that UV-B radiation can suppress nutrient uptake, bleaching pigment, inhibiting photosynthesis, and growth and alter biochemical compositions (Häder et al. 2007). In nature, *N. flagelliforme* is exposed to solar radiation and is therefore susceptible to the effects of UVR (Gao and Ye 2007). However, cyanobacteria are believed to have originated during the Precambrian era (between 2.8 and 3.5×10^9 years ago), when the ozone shield was absent, and thus faced extremely high levels of UVR (Ferroni et al. 2010). This presumably caused evolutionary pressure, eventually leading to the organism developing efficient UV-protective mechanisms (Sinha and Häder 2002). Many studies have shown that cyanobacteria possess efficient defense mechanisms for counteracting the harmful effects of UVR, such as behavioral avoidance, repair of UV-damaged DNA by photoreactivation and excision repair, restoration of PSII by increasing the turnover rate of D1 and D2 protein, and accumulation of carotenoids and detoxifying enzymes or radical quenchers that provide protection by scavenging harmful radicals or oxygen species (Xiong 2001; Qiu et al. 2003; Häder et al. 2007). Recently, Ferroni et al. (2010) found that *N. flagelliforme* is rich in mycosporine-like amino acids (MAAs) (32.1 mg g^{-1} DW) and scytonemin. These screening pigments must be responsible for the observed insensitivity of its photosynthesis to UVR (Fig. 4b). Nevertheless, UVR was shown to induce a significant decrease in the alkaline phosphatase activity after 2- and 3-h exposures and to decrease the effective quantum yield of *N. flagelliforme* after 3 h (Fig.4a, b). Since phosphatase enzyme in cyanobacteria is located in the outer surface of the membrane, cell wall, or its immediate surroundings (Grainger et al. 1989; Turner et al. 2001; Štrojsová et al. 2003), therefore, this enzyme may be easily damaged by UVR. Although the UV-absorbing compounds (UVAC, i.e., MAAs and scytonemin) are abundant in this organism, most of MAAs are concentrated in the glycan sheath in the form of oligosaccharide-linked compounds (Ferroni et al. 2010), while scytonemin is normally located in the extracellular polysaccharide sheath of the cyanobacteria (Sinha and Häder 2002, 2008). In other words, UVR can easily reach the alkaline phosphatase enzyme before it can be absorbed by the UVAC. On the other hand, APase had the ability to quickly recover the activity upon rehydration when there is enough moisture, such as morning dew. This may allow the cells to take up sufficient phosphorus before the enzyme gets affected by the solar UVR. Thus, the rapid recovery of APase on rehydration reflects an important ecological strategy for this organism to survive in the arid areas.

The decrease in enzyme activity upon UVR exposure can be due to the combined effect of photodegradation of the enzyme, inactivation of the active center, and reduced enzyme production, which may not have been evident in this study because the UV exposure was maintained for only 3 h. APase, similar to many other proteins, shows peak absorption of solar radiation at a wavelength of 270–290 nm (Scully et al. 2003). Therefore, photodegradation of the enzyme may be directly caused by the high-energy UVB. Although the absorption of APase is minimal or nil in the UV-A range, APase activity was significantly reduced by UV-A exposure. This UV-A-induced decrease in APase activity may have been caused indirectly by reactive oxygen species (ROS) production triggered in response to UV-A exposure. The concentration of ROS represents the balance between UVR-induced ROS production and ROS scavenging. Energy is essential for effective scavenging mechanisms (He and Häder 2002). However, energy supply may decrease because of the direct inactivation of energy-related enzyme(s) and decrease in photosynthesis (Fig. 4b), which is one of the main sources of energy. The exact mechanism underlying the effect of ROS on APase is not clear. It is possible that ROS, such as H_2O_2, oxidize free Fe(II) to form Fe(III) and bind to the enzyme to inactivate it. Algal P uptake has been shown to decrease in cell exposure to UVR in laboratory experiments. However, to our knowledge, the mechanism for this has not been fully investigated. Our study indicated that decreases in P uptake may in part be caused by decrease labile P availability because of lower APase activity under UV exposure.

In conclusion, the terrestrial cyanobacterium *N. flagelliforme* demonstrated higher activity of the AP with increasing temperature and pH levels, showing the maximal values at 45 °C and pH11, respectively. The UV experiment indicated that UVR irradiation may cause detrimental effect on the phosphatase activity that is vital for nutrient cycling of this organism. However, the rapid recovery of APase in the early morning may let the cell absorb enough P before it was destroyed by the UV radiation.

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