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Enhanced utilization of organic phosphorus in a marine diatom *Thalassiosira weissflogii*: A possible mechanism for aluminum effect under P limitation

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ABSTRACT

Although many studies have reported the aluminum (Al) impacts on freshwater organisms and terrestrial plants in acidic and neutral pH media, little information is available on the effects of Al on organisms in the alkaline seawater. In this study, the Al effects on marine phytoplankton were investigated by growing the axenic diatom *Thalassiosira weissflogii* in seawater media amended with varied nutrient levels. Under phosphorus (P) limited conditions, Al enrichment resulted in an enhanced diatom growth and higher biomass accumulation, as well as the maintenance of high diatom biomass during the stationary phase. The diatom displayed higher cellular alkaline phosphatase activity (APA) under Al-enriched and P-limited conditions, which was responsible for the increased uptake of dissolved organic phosphorus (DOP). Lower dissolved APA was observed in the Al-enriched culture. In contrast, Al addition did not change the phosphate speciation in seawater or the diatom uptake of dissolved inorganic P (DIP) at low concentration, but instead made the diatoms uncompetitive under high DIP conditions. The results strongly indicated that Al treatment increased the proportion of the diatom cellular APA and their utilization efficiency of DOP, which may partly account for the beneficial effects of Al on the diatom under P-limited conditions. It is thus likely that Al may influence the ocean carbon cycling by promoting the phytoplankton utilization of DOP.

1. Introduction

Aluminum (Al) is the most abundant metallic element in the earth's crust, and is ubiquitous in the environment. Although Al has been regarded as a toxic element to a number of organisms including terrestrial plants (Osaki et al., 1997), freshwater biota and even humans (Macdonald and Martin, 1988; Gensemer and Playle, 1999; Yokel, 2000), the enhanced growth of terrestrial plants induced by Al has also been reported (reviewed in Foy, 1984). In contrast to these earlier substantial reports on the Al effects on biota in the acidic and neutral pH media, little is known regarding the effects of Al on organisms in alkaline seawater.

In alkaline seawater, Al hydroxides including $Al(OH)^0_3$ and $Al(OH)^-_4$ are the predominant forms of dissolved Al. Speciation of Al in alkaline seawater is greatly different from those in acidic and neutral pH freshwater media (Macdonald and Martin, 1988). Recent studies showed that the phytoplankton cellular accumulation of Al could not be solely explained by the free Al speciation when pH was higher than 6.5 (Crémazy et al., 2013a, 2013b). These results then led to speculation that phytoplankton could not only take up Al^{3+} but also the Al hydroxides. Clearly, the distinct Al speciation in alkaline seawater suggested that its influence on marine organisms may be different from those observed in freshwater organisms.

Earlier studies on Al and marine biota interaction mainly focused on the scavenging effects of biological activity on the distribution of Al in seawater, but seldom dealt with the Al influences on marine organisms. Many studies found that marine plankton could take up and/or absorb Al, and scavenge dissolved Al in seawater (e.g. Bostrom et al., 1974; Mackenzie et al., 1978; Moran and Moore, 1988; Saçan and Balcioglu, 2001; Ren et al., 2011; Dammshäuser et al., 2013; Li et al., 2013). Over fifty years ago, Menzel et al. (1963) found that the addition of Al to water collected from the Sargasso Sea had a stimulatory effect on the productivity of phytoplankton (particularly diatoms) in the water, if nitrate and phosphate were simultaneously added, and sufficient silicate was present. Stoffyn (1979) reported that Al had a stimulatory effect on the growth of a marine diatom *Skeletonema costatum*. Later, Vrieling et al. (1999) showed that Al enrichment increased the growth of a pennate diatom *Navicula salinarum* but not of a centric species *Thalassiosira weissflogii*. Saçan et al. (2007) reported that Al had a stimulatory effect at low concentration (500 μg l^{-1}) and an inhibitory effect at high concentration (>4000 μg l^{-1}) on the growth of *Dunaliella*

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tertiolecta. Other studies documented the incorporation of Al in dead and living diatom frustule (Gehlen et al., 2002; Koning et al., 2007). A most recent study reported the high tolerance to Al by 11 marine organisms (including four phytoplankton species) representing 6 taxonomic groups (Golding et al., 2015). All these findings implied that Al was likely involved in the marine biogeochemical cycles by affecting marine phytoplankton growth, but very few studies examined the Al effects on marine phytoplankton growth and the underlying mechanisms of such effect, if any.

Nutrient utilization is one possible mechanism for the beneficial effects of Al on plant growth. The possible roles of Al in the enhanced plant growth including the increased iron availability, promotion of phosphorus (P) uptake, and protection against copper, manganese and P toxicity were first reported for different plant genotypes and growth media (reviewed in Foy, 1984). Then, alleviation of H^+ toxicity (Kinraide, 1993) and increase of nitrogen, P and potassium uptake (Osaki et al., 1997) by plant roots were considered to be the general mechanisms for the stimulatory effects of Al on terrestrial plant growth in acidic media. Whether or not Al affects phytoplankton utilization of nutrients like P in alkaline seawater is unknown.

In the present study, the diatom growth under different Al enrichments and P-limited conditions was firstly quantified, and then the P (both inorganic P and organic P) utilization under Al-enriched conditions was measured. The present study provided evidence that the sustained diatom growth by Al was likely mediated by the availability of nutrients such as DOP.

2. Materials and methods

In order to examine the Al effects on marine phytoplankton growth and understand the underlying mechanisms, axenic cultures of a marine diatom *T. weissflogii* (CCMP1336) were grown in seawater media with varied nutrient levels (including low nutrient, and P-deficient) and with/without Al amendment, and the growth of the cells was monitored. Furthermore, the Al influences on DIP speciation and diatom utilization of DIP and DOP in seawater were examined. A range of Al concentrations (40 nM, 200 nM, 2 μM and 20 μM) were tested in this study. These four concentrations corresponded to the levels in ocean surface impacted by high dust deposition (40 nM), the upper range of dissolved Al in natural seawater (200 nM), the level in riverine waters of the estuary (2 μM), and the level approximate to the solubility of Al in seawater (20 μM), respectively (de Jong et al., 2007; Brown and Bruland, 2009; Golding et al., 2015). Golding et al. (2015) estimated that Al at levels of 24 μg/l (~0.89 μM) were safe for 95% of marine organisms. The Al salt used for amendment had a purity of 99.999% based on trace metals analysis (Sigma-Aldrich 563,919); therefore any possibility of other metal addition into the seawater medium was considered to be minimal if existent.

2.1. Seawater media and diatom incubation

Several incubation experiments were firstly conducted in media using seawater collected from an oligotrophic basin in the South China Sea, and aged seawater collected from the coast off eastern Hong Kong. For the first experiment, the oligotrophic seawater with low levels of dissolved inorganic nutrients (0.15 μM nitrate plus nitrite and 3.7 μM silicate) was spiked with sufficient silicate (42 μM), and was then enriched with 1) Al (Al, 2 μM $AlCl_3$), 2) nitrate and phosphate (NP, 1 μM $NaNO_3$ and 0.1 μM NaH_2PO_4), 3) nitrate, phosphate and Al (NPA, 1 μM $NaNO_3$, 0.1 μM NaH_2PO_4 and 2 μM $AlCl_3$), and 4) nothing as control. The diatom *T. weissflogii* in exponential phase was incubated for three days in the oligotrophic seawater medium spiked with 42 μM of silicate. The treated *T. weissflogii* was inoculated to experimental bottles to reach an initial cell density of 4.6×10^3 cells ml^{-1}.

For the other experiments, the aged seawater (with 0.37 μM nitrate plus nitrite, 4.3 μM silicate and non-detectable phosphate) was enriched according to modified f/2 recipes (https://ncma.bigelow.org/node/79) including the f/200 recipe, and the f/20 recipe with modified phosphate and/or silicate concentrations. Two different concentrations of phosphate (0.36 μM and 3.62 μM corresponding to the f/200 and f/20 recipes, respectively) were added into the media to achieve the P-deficient and -sufficient conditions, respectively. Silicate (106 μM) was added into the media to achieve the f/20 medium with modified silicate. $AlCl_3$ (20 μM) was added to the medium for the treatment but not for the control.

For all the above experiments, diatom cells were grown in 500-ml polycarbonate bottles. Three replicates were prepared for each treatment. The bottles were incubated under a constant temperature of 24 °C, with a light:dark cycle of 14 h:10 h and light intensity of 128 μmol photons m^{-2} s^{-1}. All the cultures were mixed gently (without disrupting the cells) twice a day. At the beginning of the cultures the pH in the media was adjusted to 8.1.

Cell abundance and/or chlorophyll *a* (Chl *a*) were monitored during the incubation. Diatom cell abundance was quantified by the microscope or by using a Becton Dickinson FACSCalibur cytometer with a 488-nm laser (with yellow-green fluorescent beads 10 μm in diameter, Polysciences, Inc. as the internal standard). The red fluorescence signal (FL3) was used as the indicator for cellular chlorophyll, and the side scatter signal (SSC) was used as the indicator for cell size (Tzur et al., 2011). To determine the Chl *a*, 5 ml of the diatom cells was filtered on Whatman GF/F filter. Chlorophyll *a* was extracted by dipping the filters into 90% acetone at −20 °C in darkness for 24 h, and analyzed by fluorometry using a Turner Designs 10-AU Fluorometer (Parsons et al., 1984).

2.2. Diatom growth under P-limited and P-sufficient conditions in Aquil medium

The influences of varied concentrations of Al on diatom growth were further tested under P-limited conditions in modified Aquil medium with only 0.2 μM phosphate. Chemicals and protocols for the preparation of the medium suggested by Sunda et al. (2005) were followed. Specifically, natural seawater collected from the oligotrophic basin of the South China Sea was used for the Aquil medium. All the stock solutions of macronutrients, vitamins, trace metals and Al were prepared in a 100-class clean room. The macronutrients were Chelex-treated in the 100-class clean room.

In this experiment, a range of Al addition, including 40 nM, 200 nM, 2 μM and 20 μM, was used. Three replicates were prepared for each treatment. All the bottles were set overnight before the inoculation of *T. weissflogii* cells in exponential phase to reach an initial density of 900 cells ml^{-1}. The diatom was cultured under a continuous light regime with a light density of 120 μmol proton m^{-2} s^{-1} at 20 °C. Diatom cell abundance and cell volume in the media was measured by a Beckman Coulter Counter (Z2). In vivo Chl *a* (fluorescence) was measured by using the Tuner Designs Trilogy Fluorometer. The pH was measured by using the Thymol-blue method (Zhang and Byrne, 1996). All the sampling was conducted in an aseptic, dust-free 100-class clean Laminar flow cabinet.

The diatom growth rates on the basis of cell abundance, cell volume and in vivo Chl *a* were calculated both in the exponential phase and in the early stationary phase (from the end of the exponential phase to the start of the decay phase).

To examine the influences of Al on diatom growth under P-sufficient conditions, *T. weissflogii* cells in the exponential phase was inoculated in the Aquil medium with 10 μM phosphate and varied amounts of Al to set the control treatment and the Al-enriched ones with final Al concentrations of 40 nM and 200 nM, respectively. Three replicates were prepared for each treatment. The diatom was cultured and monitored under the same conditions as described above.