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Protective function of nitric oxide on marine phytoplankton under abiotic stresses

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ABSTRACT

As an important signaling molecule, nitric oxide (NO) plays diverse physiological functions in plants, which has gained particular attention in recent years. We investigated the roles of NO in the growth of marine phytoplankton *Platymonas subcordiforms* and *Skeletonema costatum* under abiotic stresses. The growth of these two microalgae was obviously inhibited under non-metal stress (sodium selenium, Na_2SeO_3), heavy metal stress (lead nitrate, $Pb(NO_3)_2$), pesticide stress (methomyl) and UV radiation stress. After the addition of different low concentrations of exogenous NO (10^{-10}–10^{-8} mol L^{-1}) twice each day during cultivation, the growth of these two microalgae was obviously promoted. Results showed that NO could relieve the oxidative stresses to protect the growth of the two microalgae. For different environmental stress, there is a different optimum NO concentration for marine phytoplankton. It is speculated that the protective effect of NO is related to its antioxidant ability.

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Introduction

Nitric oxide (NO) is both a gaseous free radical and an important messenger that plays important roles in diverse physiological processes. In animals, NO functions include modulating cardiovascular disease, antiplatelet activity, immunoregulation, antiulcer effects and so on [1–3]. NO is an important signaling molecule and ROS (reactive oxygen species) scavenger in animals with dual functions. One is that a low concentration of NO is protective by scavenging superoxide radicals (O^{2-}) and lipid free radicals (R), interrupting the process of lipid peroxidation and inducing the expression of antioxidant enzymes. The other is that a high concentration of NO can react with (O^{2-}) to form peroxynitrite, which can destroy the structure and function of biological macromolecules [4–7].

NO has also become an increasingly popular target of investigation in plants since NO has been implicated in disease resistance [8,9]. In plants, NO acts as a signaling molecule in numerous processes, including development, growth, defense [7,10], germination [11], and stomatal closure [12]. Kopyra and Gwóźdź [13] showed that NO counteracted the inhibitory effect of heavy metals (Pb, Cd) and salinity on the root growth of *Lupinus luteus*. It was also reported to weaken the toxicity of ROS generated by diquat or paraquat (PQ) in potato and rice [5,14]. NO was regarded as plant growth regulators or a plant hormone [10,15].

NO has attracted significant attention from scientists in the study on the phytoplankton in recent years. It was reported that algae *Anabaena doliolum* and *Scenedesmus obliquus* could emit NO when nitrate (NO_3^-) or nitrite (NO_2^-) was supplied in darkness [16,17]. Sakihama et al. [18] showed that green alga *Chlamydomonas reinhardtii* could produce NO in the dark when nitrite (NO_2^-) was provided. Tischner et al. [19] advanced that mitochondrial electron transport as a source for NO in the unicellular green alga *Chlorella sorokiniana*. Zhang et al. [20] studied the effects and chemical characteristic of NO on the growth of marine microalgae and found that different concentrations of NO could promote or inhibit the growth of microalgae, then put forward the view of "NO threshold". At the same time, Zhang et al. [21] reported NO was detected in both the lab and the alga culture pond of Daya Bay in China, with the concentrations of 10^{-9}–10^{-8} mol L^{-1}. Kim et al. [22,23] elucidated that *Chattonella marina* and *Heterosigma adashiwo* produced NO under normal growth conditions and suggested that a NOS-like enzyme was mainly responsible for NO generation in *C. marina*. Singh et al. [24] demonstrated the antioxidative role of NO on copper toxicity to a chlorophycean alga, *Chlorella*. Chen et al. [25] considered that UV-B appeared to be a strong inducer of NO production, exogenously added NO and reductants protecting the green alga against UV-B induced oxidative damage.

Abbreviations: NO, nitric oxide; ROS, reactive oxygen species; NOS, nitric oxide synthase; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; POD, peroxidase; GPX, guaiacol peroxidase; GR, glutathione reductase; ABA, abscisic acid.

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With increasing levels of marine environmental pollution, marine primary production becomes an important issue of concern. Since NO has been proved to be capable of alleviating some consequences of oxidative stress, what role does NO play under abiotic stresses in marine phytoplankton such as non-metal stress (sodium selenium, Na_2SeO_3), heavy metal stress (lead nitrate, $Pb(NO_3)_2$), pesticide stress (methomyl) and UV radiation stress? Our study focused on the protective effects of NO on the growth of *Platymonas subcordiforms* and *Skeletonema costatum* cultured in the laboratory under the above four abiotic stressors, which may contribute to the research of NO marine biogeochemical processes.

Materials and methods

Preparation of NO solution

NO standards were prepared by making serial dilutions of saturated NO solutions. Milli-Q water (2 mL) was bubbled with high purity N_2 gas (99.999%, Qingdao Heli Industry Gas Center, China) at a speed of 10 mL min^{-1} (6 mm diameter tube) for 30 min to remove oxygen. The solution was then bubbled with high purity NO gas (99.9%, Dalian Date Gas Ltd, China) for 30 min. The concentration of a saturated NO solution is 1.4×10^{-3} mol L^{-1} [26,27]. Standards were freshly made for each experiment and kept in a glass flask with a rubber septum. Dilutions of the saturated solution were made using Milli-Q water.

Phytoplankton cultivation

The microalgae cultivated in the experiment were *P. subcordiforms* and *S. costatum*, which were obtained from the Marine Microalga Research Laboratory, Ocean University of China. The seawater used for microalgae cultivation was collected from the Yellow Sea, filtrated immediately with a 0.45 m acetate cellulose membrane (Millipore, USA), and sterilized by LDZX-II Autoclaves Sterilizer (Shanghai Shenan Medical Instrument Factory, China) for 20 min. The procedure of phytoplankton culture was as follows: Microalgae were transferred into Erlenmeyer flasks (soaked in sulphuric acid beforehand and then rinsed thoroughly with distilled water) with F/2 medium [28] and cultured till the exponential growth phase. The algae were inoculated at the ratio of 31, i.e., each bottle contained 150 mL culture medium and 50 mL alga solution, and was put into an Illumination Cultivating Box (GXZ-380B, Jiangnan Instrument Factory, Ningbo, China), with a lighting rhythm of 14 light:10 dark and a light irradiance of 4500 lux, at 20 ± 0.5 °C.

Experimental design

In order to investigate abiotic stresses for *P.* subcordiforms and *S. costatum*, some preliminary experiments were conducted. After inoculation, the prepared solutions of sodium selenium (Na_2SeO_3), lead nitrate ($Pb(NO_3)_2$) and methomyl were added to the suspension of microalgae during the exponential growth period, respectively. It was found that 10^{-4} mol L^{-1} of Na_2SeO_3 showed inhibition influence on the growth of *P.* subcordiforms while 10^{-5} mol L^{-1} of Na_2SeO_3 exhibited inhibition effect for *S. costatum*. It was also found that the addition of 10 mg L^{-1} $Pb(NO_3)_2$ has inhibition influence on the growth of both *P.* subcordiforms and *S. costatum*. The concentration of 2 mg L^{-1} of pesticide methomyl presented inhibition effect on the growth of these two microalgae.

In order to investigate the effect of NO on marine phytoplankton growth, a preliminary experiment for *P. subcordiforms* and *S. costatum* was carried out. Some solutions of NO were added at certain time twice a day. Initial concentrations were set at 0 (control), 14, 1.4, 0.14, 0.014, 0.0014 and 0.00014 μmol L^{-1}, respectively. It was found that the growth of these two algae was obviously promoted when 0.14–14 nmol L^{-1} of NO was added [20]. Furthermore, the measured NO concentration was 10^{-9}–10^{-8} mol L^{-1} in both the lab and the alga culture pond [21].

Based on the results above, adding Na_2SeO_3 to the cultivation of *P. subcordiforms* made sure that the concentration of Na_2SeO_3 was 0 or 10^{-4} mol L^{-1}, and 0 or 10^{-5} mol L^{-1} for *S. costatum*. Initial $Pb(NO_3)_2$ concentrations were set in the culture medium at 0 or 10 mg L^{-1} for these two algae, respectively. The addition concentration of methomyl for these two algae was 0 or 2 mg L^{-1}. The UV-B stress was designed as that after UV-B radiation (HR 1×18w, Xinghui Electric Instrument Factory, China) for 10 min, microalga was cultured in darkness for 24 h, then cultured in experimental condition.

For every stress condition experiment, NO was added twice a day (10am, 3 pm) to the suspension of microalga in every bottle, the addition concentration was 0, 1.4×10^{-10}, 1.4×10^{-9} and 1.4×10^{-8} mol L^{-1}, respectively.

Measurement

Culture cell densities were determined with a fluorescence spectrophotometer (Type F-4500: Hitachi, Japan) with a 1 cm cuvette. With Mili-Q water as a control under fluorometric determination conditions (λ_{ex} = 436 nm, λ_{em} = 670 nm), the fluorescence intensity of microalga cell was measured at a fixed time every other day. The concentrations of the microalga cell and the fluorescence showed a good linear correlation ($R^2 > 0.95$). The growth curve of each microalga sample was finally obtained by plotting the cell number of microalga against the cultivation time.

Statistical analysis

All of the data were expressed as mean ± S.D. (standard deviation). A one-way ANOVA was used to determine the effects of sodium selenium, lead nitrate, methomyl or UV-B and different concentrations of NO on the growth of *P. subcordiforms* and *S. costatum*.

Results

Effects of NO and sodium selenium on the growth of Platymonas subcordiforms and Skeletonema costatum

We added sodium selenium with a concentration of 10^{-5} mol L^{-1} to the culture medium of *P.* subcordiforms at the beginning and found that sodium selenium of this concentration could not inhibit the growth of *Platymonas subcordiforms.* Then we increased the sodium selenium concentration to 10^{-4} mol L^{-1}. Compared with the control, the cell density of *P. subcordiforms* decreased by 13.0% on the fourth day from the cultivation and on the sixth day, the cell density of *P. subcordiforms* declined by 6.5%, as shown in Fig. 1a. During the following days, sodium selenium with the concentration of 10–4 mol L^{-1} not only could not inhibit the growth of *Platymonas subcordiforms*, but also could promote the growth of *Platymonas subcordiforms*. When 1.4×10^{-10}–1.4×10^{-8} mol L^{-1} of the NO solution was added twice a day (10am, 3 pm), there were no obvious protective effects against sodium selenium stress for *P. subcordiforms* (Fig. 1c). When 10^{-5} mol L-1 sodium selenium was added to the culture medium of *S. costatum*, 1.4×10^{-10}, 1.4×10^{-9} or 1.4×10^{-8} mol L^{-1} NO was added twice a day to investigate the effect of NO, as shown in Fig. 1b. The experimental results showed that 10^{-5} mol L^{-1} sodium selenium could inhibit the growth of *S. costatum*. When different concentrations of NO were added, NO could relieve the sodium selenium stress

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