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Evidence for the mutual effects of dimethylsulfoniopropionate and nitric oxide during the growth of marine microalgae

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ABSTRACT

Dimethylsulfoniopropionate (DMSP) and nitric oxide (NO) in marine microalgae are considered as two important compounds involved in a variety of physiological functions. We examined the NO responses and the growth of *Isochrysis galbana Parke* and *Gymnodinium sp.* when supplemented with different concentrations of DMSP solutions in the cultures. Production of DMSP and dimethylsulfide (DMS) in *Amphidinium carterae* and *Emiliania Huxleyi* was investigated after the addition of NO donor sodium nitroprusside (SNP) and NO solution to algal media. The release peaks of NO were observed in cell suspensions of *I. galbana Parke* and *Gymnodinium sp.* immediately after the injection of DMSP solutions. The growth of these two microalgae was found to be significantly promoted or inhibited caused by exogenous DMSP. There was a decrease of DMSP concentrations in algal cultures within 24 h, accompanied with an increase in DMS, due to the effect of NO. The results provided direct evidence to confirm that there exist mutual effects of DMSP and NO during the growth of marine microalgae, which is speculated to be related to their roles as signaling molecules in planktonic communities.

1. Introduction

As a major component of organic sulfur in the ocean, dimethylsulfoniopropionate (DMSP) plays a key role in the global sulfur cycle. DMSP production is a widespread process in phytoplankton communities but its magnitude varies greatly among taxa. Raina et al. also reported DMSP biosynthesis by an animal [1,2]. The cleavage of DMSP can release dimethylsulfide (DMS), which is the dominant volatile sulfur compound in surface waters of the ocean and contributes 17.6–34.4 TgS per year to the atmosphere [3]. DMS is thought to significantly affect the global climate through the formation of cloud condensation nuclei (CCN) that influence cloud droplet size and cloud albedo [4,5], though this hypothesis has been challenged recently [6]. DMS can also contribute to the acidity of atmospheric particles and rainfall [7,8]. In addition to its well-known role as the precursor of DMS, DMSP is recognized as a significant part of the sulfur and carbon fluxes through marine microbial food webs [9–12], and as an intracellular osmolyte in marine algae, a cryoprotectant in polar algae and a grazing deterrent via its cleavage to acrylate [13,14]. DMSP and its degradation products (DMS, acrylate, dimethylsulfoxide, and methane sulfinic acid) could scavenge hydroxyl radicals and other reactive oxygen species (ROS), thus the antioxidant system may be partially regulated by the enzymatic cleavage process [15,16]. So far, most DMSP and DMS research has been on their spatiotemporal distributions in the marine environment. Less effort has been devoted to how DMSP and DMS are produced and the factors influencing DMSP synthesis in phytoplankton [1].

Nitric oxide (NO) is a major constituent of NOx and its reaction with oxygen in the atmosphere leads to the formation of nitrogen dioxide (NO_2), which causes acid rain, the depletion of the ozone layer and harmful effects on human health. Because of its high reactivity (having a lifetime of a few seconds) and free diffusion across membranes, NO is considered as an important inter- and intracellular signaling molecule in plants as well as animal systems [17–19]. NO had key effects on plant growth and development, including seed germination, root elongation, leaf senescence, stomatal closure, disease resistance and the responses to both biotic and abiotic stresses in plants [20–27]. NO even acts as an antioxidant and quenches ROS by regulation the activity of antioxidant enzymes including superoxide dismutase (SOD), guaiacol peroxidase (GPX), catalase (CAT) and glutathione reductase (GR) to scavenge ROS, and then provides protection against cellular injury [28–33]. However, it is usually toxic at high NO concentrations.

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Since Mallick et al. pointed out that *Anabaena doliolum*, a cyanobacterium, was a better NO producer than *Scenedesmus* and *Synechoccocus* [34], the increasing researches have appeared concerning NO in algae and cyanobacteria [35–42]. It has been suggested to act as a signaling molecule and play a regulatory role during the growth of microalgae. The microalgae not only could present response for exogenous NO, but also could yield low concentrations of NO [43–50]. It is worth noting that a NO peak usually occurred just before the cell density reached the maximum [51]. The ROS metabolism in algal cells presented an increasing trend with increased NO concentration, and the SOD activity and malondialdehyde (MDA) contents also had corresponding changes in algae [29,52]. In the ocean ecosystem, the yielding NO can occur through photolysis, nitrification and denitrification processes [53–55]. However, the mechanism of NO biosynthesis and the role of NO in microalgae are still unclear.

Based on the common characteristics of multifunction and relationship with ROS between NO and DMSP in algae, we here aim to shed light on the mutual effects of NO and DMSP in marine microalgae, which is contributive to the further understanding of the physiological roles of NO and DMSP in the marine ecosystem.

2. Materials and methods

2.1. Chemicals

In the present study, we performed experiments in algae with a well-known NO donor, sodium nitroprussiate (SNP), which was purchased from Sigma-Aldrich Corporation, USA. DMSP hydrochloride was provided by Research Plus, Inc. (99.9995%, Bayonne, NJ, USA). Pure NO gas (99.9%) was obtained from Dalian Date Gas Ltd., China. High purity nitrogen gas (99.999%) was purchased from Heli Industrial Gas Center, Qingdao, China. All solutions were prepared using ultrapure water (18.2 MΩ cm, Millipore Company, USA).

2.2. Algal cultivation

Isochrysis galbana Parke and *Gymnodinium sp.*, provided by the Marine Pollution Eco-chemistry Laboratory, Ocean University of China, were cultivated in Key Laboratory of Marine Chemistry Theory and Technology, Qingdao. All cultures were axenic and unialgal. Seawater was collected from the Yellow Sea, filtrated immediately with a 0.45 μm acetate cellulose membrane (Millipore), and sterilized by LDZX-II Autoclaves Sterilizer (Shanghai Shenan Company, China). All glassware used in the experiments was soaked in HCl for at least 24 h, washed with ultrapure water, and then autoclaved (LDZX-50KBS, Shanghai Shenan Medical Instrument Factory). For each of the cultures, 150 mL f/2 medium [56] was added to a 250 mL Erlenmeyer flask, and the strain was inoculated by adding 50 mL of the stock culture in the exponential growth phase. All cultures were kept at 20 ± 2 °C in an illumination incubator (GXZ-380B, Ningbo Jiangnan Instrument Factory, China) under the same light conditions (12 h light:12 h dark cycle) with an average photon flux density of 74 μmol $m^{-2}s^{-1}$. To avoid algal cell precipitation, flasks were shaken three times a day. The growth of microalgae was monitored by Cover-15 microscope (Olympus, Japan) during the cultivation [50].

Axenic algal cultures, *Amphidinium carterae* and *Emiliania Huxleyi*, were purchased from the Provasoli-Guillard Center for the cultivation of Marine Phytoplankton and cultivated in the laboratory at College of Environmental Science and Forestry, State University of New York. Seawater was collected from coastal waters of New York City and filtrated immediately through a 0.45 μm acetate cellulose membrane. Algae were grown in f/2 media under an average photon fluorescent density of 120 μmol $m^{-2}s^{-1}$ (14 h light: 10 h dark). All cultures were maintained at 22 ± 1 °C in 125 mL polycarbonate Erlenmeyer flasks. Algal cell densities were measured with a Beckman-Coulter Z2 Particle Counter using 1% NaCl buffered with 50 mmol·L^{-1} sodium phosphate (pH 7.8) as the electrolyte diluent. Cells were diluted by a factor of 50 (200 μL of culture into 10 mL of diluent). The buffered saline solutions were filtered through a 0.2 μm Polycap™ 36 AS filter (Whatman) prior to use [57].

The red tide species *I. galbana Parke, Gymnodinium sp., A. carterae*, and *E. Huxleyi* are widely distributed in estuarine, coastal, and marine waters. These prymnesiophytes and dinoflagellates are major DMSP and DMS producers in seawater, and more sensitive to NO than food alga. We used these species to study on the mutual effects of NO and DMSP in marine microalgae.

2.3. Electrochemical detection of nitric oxide

Electrochemical detection of NO was performed using an ISO-NO Mark II NO determination instrument, connected to an ISO-NOPMC microsensor (World Precision Instruments (WPI), Inc., Sarasota, FL, USA). The analog signals from the ISO-NO Mark II NO meter were digitized using a DUO18 two-channel data-acquisition system (WPI, Inc.) connected to a computer [49]. The detection limit was 0.42 nmol·L^{-1} and the relative standard deviation was 6.3%. A total of 10 mL medium in a glass vial with a rubber septum was bubbled with high purity nitrogen gas for 30 min to remove oxygen. Aliquots of standard saturated or diluted NO solutions were injected with gas-tight syringe, then a relationship between the response current and NO concentration was obtained. Data were recorded under constant stirring conditions of solution at room temperature [43,51,58].

2.4. Sulfur analyses

DMS was removed by sparging for 2 min with UHP He (200 mL·min^{-1}) and concentrated in a Teflon cryotrap cooled by liquid nitrogen. When the Teflon tubing was warmed by near-boiling water, the trapped DMS was swept into a Shimadzu gas chromatograph (GC-14A, Japan) equipped with a 2.4 m long × 3.2 mm inner diameter (i.d.) Teflon column packed with Chromosil 330 (Supelco) and a flame photometric detector (FPD). The column was held at 60 °C, with the injector and detector both set at 225 °C. Under such conditions, DMS was eluted at 1.4 min [57]. Standards for DMS analysis were made by sparging known amounts of DMS (produced from base hydrolysis of DMSP) in the same manner as samples. Standards were maintained at the same temperature, volume, and approximate ionic strength as samples. The analytical precision was on the order of ± 5% at the 15 nmol·L^{-1} range. DMSP was measured as DMS analysis after the alkaline cleavage to DMS at a 1:1 stoichiometry [59].

2.5. Microalgae test of DMSP on the production of NO

Aliquots of 10 mL culture sample of *I. galbana Parke* at the cell density of 342.1 × 10^4 cell·mL^{-1} during the exponential growth phase were bubbled in a 17 mL serum glass vial with high purity nitrogen gas to remove oxygen. Then 50 or 100 μL of 15 mmol·L^{-1} DMSP solution (corresponding to 74.6 or 149 μmol·L^{-1} DMSP of final concentration) was injected to the deoxy-medium via syringe and the response current by the NO analyzer was observed. As a control, 50 or 100 μL of Milli-Q water was also injected into the same culture medium and the response was recorded. For the experiments with *Gymnodinium sp.* at the cell density of 43.4 × 10^4 cell·mL^{-1}, 250 or 500 μL of 15 mmol·L^{-1} DMSP solution were added, that is, the final concentrations were 167 or 335 μmol·L^{-1}, and the same volumes of Milli-Q water served as controls.