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Research paper

Membrane lipid profiles of coral responded to zinc oxide nanoparticle-induced perturbations on the cellular membrane

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ABSTRACT

Zinc oxide nanoparticles (nZnOs) released from popular sunscreens used during marine recreation apparently endanger corals; however, the known biological effects are very limited. Membrane lipids constitute the basic structural element to create cell a dynamic structure according to the circumstance. Nano-specific effects have been shown to mechanically perturb the physical state of the lipid membrane, and the cells accommodating the actions of nZnOs can be involved in the alteration of the membrane lipid composition. To gain insight into the effects of nanoparticles on coral, glycerophosphocholine (GPC) profiling of the coral *Seriatopora caliendrum* exposed to nZnOs was performed in this study. Increasing lyso-GPCs, docosapentaenoic acid-possessing GPCs and docosahexaenoic acid-possessing GPCs and decreasing arachidonic acid-possessing GPCs were the predominant changes responded to nZnO exposure in the coral. A backfilling of polyunsaturated plasmanylcholines was observed in the coral exposed to nZnO levels over a threshold. These changes can be logically interpreted as an accommodation to nZnOs-induced mechanical disturbances in the cellular membrane based on the biophysical properties of the lipids. Moreover, the coral demonstrated a difference in the changes in lipid profiles between intra-colonial functionally differentiated polyps, indicating an initial membrane composition-dependent response. Based on the physicochemical properties and physiological functions of these changed lipids, some chronic biological effects can be incubated once the coral receives long-term exposure to nZnOs.

1. Introduction

Zinc oxide nanoparticles (nZnO) are common constituents of sunscreens and cosmetics that are unwittingly released to the aquatic environment (Osmond and Mccall, 2010). The levels of nZnOs in terrestrial surface water have been predicted at hundreds of μg/L and are expected to continually elevate due to the widespread application (Boxall et al., 2007; Ma et al., 2013). The toxicity of nZnO was demonstrated on a variety of freshwater and saltwater organisms, such as microalgae, crustaceans, mollusks and fish (Ma et al., 2013; Minetto et al., 2016; Trevisan et al., 2014; Wong et al., 2010). Scleractinian corals, which form the base of coral reef ecosystems, are directly exposed to nZnO derived from the use of sunscreen during snorkeling activities in addition to other indirect pollutant sources. However, information about the biological effects of nZnO on coral is very limited. There are only a few studies that have demonstrated the toxic effects of other nanomaterials on cnidarians. For example, silver nanoparticles were shown to adversely affect the early life stages of scleractinian coral and the behaviors of jellyfish starting at 50 and 100 μg/L, respectively (Gambardella et al., 2015; Suwa et al., 2014). The Caribbean reef-building coral expels algal symbionts and increases the expression of stress proteins when exposed to titanium dioxide nanoparticles at the levels of 0.1–10 mg/L for 1-week (Jovanovic and Guzman, 2014).

The metal ion and/or particle -induced oxidative stress and cellular damages were summarized as the major phenomena driving the toxicity of metal and metal oxide nanoparticles (Ivask et al., 2014). Here, the nano-specific effects were focused due to the importance of understanding the cellular uptake and consequent toxic action of nanoparticles. It is apparent that the attachment of nanoparticles to the cellular membranes can be a critical initial process that precedes cytotoxic pathways (Ivask et al., 2014; Treuel et al., 2013). Through electrostatic

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forces, nanoparticles can interact with the components both on the membrane surface and within the membranes, such as phospholipids and proteins (Chen and Bothun, 2014). This process perturbs intermolecular interactions between the membrane components, which can lead to the membrane lipids restructuring, which changes the lipid phase behavior and the membrane organization and structure. Nanoparticles can consequently extract lipids from the membrane and translocate across the membrane through poration or invagination, causing membrane tension and leakage. As shown, nZnOs can attach to the cell surface of organisms and then cause mechanical damage resulting the membrane deformation and the morphological change of the cells and even organelles (Chen et al., 2012; Hu et al., 2010; Li et al., 2011; Peng et al., 2011; Trevisan et al., 2014; Xiong et al., 2011). Supposedly, nZnOs treatment increases the permeability of cellular membranes and depolarizes cells, partly as a result of such mechanical damage (Chen et al., 2012; Xie et al., 2011).

Cellular membranes play a major role in cell function by forming a physical barrier, regulating and mediating transmembrane transportation, and providing a matrix for multi-component assembly in metabolic and signaling pathways (van Meer et al., 2008). They are a dynamic structure that undergoes transient physicochemical changes depending on the cellular circumstances. Membrane lipids constitute the basic structural elements required to create the dynamic condition. These lipids have diverse structures of the molecule that can sustain membrane function following an environmental perturbation, as well as maintain cellular membranes with physicochemical properties that are appropriate for physiological requirements (Frolov et al., 2011; Hazel and Williams, 1990). The cellular function, adaptation or dysfunction can be therefore reflected in the variation of the molecular composition of membrane lipids. As shown, organisms that sustain membrane function by altering fatty acid composition of the membrane lipid can remain viable under stress conditions (Mykytczuka et al., 2011; Rozentsvet et al., 2012). Scleractinian coral alters the profile of membrane lipids to accommodate the cellular membrane to intracolonial functional differentiation or the conditions of oxidative stress (Tang et al., 2014; Tang et al., 2015). Nevertheless, even relatively small long-term alterations in the membrane lipid profile can elicit considerable cellular perturbation, leading to chronic effects on the fitness of organisms (Hazel and Williams, 1990; Tang et al., 2014).

Most studies on the effects of nanoparticles on organisms have characterized acute toxicities. They lack evaluation of the metabolic response of membrane lipids that can reflect the action of nanoparticles on the membrane and the chronic physiological consequences. It is essential to have insight into the molecular structures of altered membrane lipids that determine the ability of the lipid composition to adapt cell to the specific circumstance. High-throughput methods in lipidomics have made this detailed analysis possible, allowing us to systematically conduct lipid profiling with an explicit description of the molecular structure. Considering the lack of understanding of the harms and the ecological importance, in this study, membrane lipid profiling of a pocilloporid coral, *Seriatopora caliendrum*, in response to nZnOs exposure was performed using a mass spectrometry-based lipidomics method. In addition, it is hypothesized that the functionally differentiated coral polyps within a coral colony will respond to nZnOs exposure differently due to their difference in the vulnerability (Tang et al., 2015). The lipid profiling of the distal and proximal portions of coral branches was respectively carried out to contrast the difference responses to nZnOs exposure. The relevance of the membrane lipid alterations to the cellular accommodation to the disturbances induced by nZnO was explained. Altered lipid species that are critical for coral resistance and growth are further discussed.

2. Materials and methods

2.1. Exposure experiment

S. caliendrum coral colonies were collected from the coastal region of Kenting National Park, Taiwan, at depths of 8–10 m during 2011. All of the colonies were maintained and cultivated in a 7 kl flow-through aquarium with shaded ambient light ($<$ 600 μmol quanta s^{-1} m^{-2}) at the National Museum of Marine Biology and Aquarium near the collection location. The seawater temperature was maintained at 27 ± 0.5 °C by a circulatory system using thermal regulation. Coral branches of approximately equal size (~2.5 g) were severed from the colonies and incubated in the same aquarium. The coral branches were used for the exposure experiments once the wounded site had completely healed with developed packed polyps. The clade of the zooxanthellates in the corals was identified according to the internal transcribed spacer rDNA sequence (Tang et al., 2015). After the pretreatment procedure, the selected clones were sequenced, and the obtained sequences were searched for and aligned using the NCBI GenBank Database with BLAST (http://www.ncbi.nlm.nih.gov/). The results indicated that the zooxanthellates belonged to clade C (accession: KC684906 and KC684907).

The coral branches were exposed to a 40 μg/L (ca 0.6 μM)-augmented Zn^{2+} treatment and three nZnO levels being 50, 100 and 200 μg/L (ca 0.6, 1.2 and 2.5 μM) in parallel with a control group for 24 h (12 h light/12 h dark). Suspension of nZnO (cod. 544906, mean particle size 50–70 nm, Sigma-Aldrich, USA) and a stock solution of Zn^{2+} ($ZnSO_4 \cdot 7H_2O$, Merck, Darmstadt, Germany) were prepared in 100 mL filtered seawater to the concentration of 100 mg/L. The nZnO suspension was further sonicated for 60 min in an ultrasonic bath (40 kHz frequency, Mandarin Scientific, Taiwan) with shaking prior to direct dosing in the experiment. There were 7 replicate coral branches derived from different colonies in the control and treatment groups. Each coral branch was maintained in an individual glass bottle containing 1.8 L filtered seawater derived from the same source as the aquarium in which the coral was cultivated. During the experiment, each glass bottle was gently aerated and placed in the same artificial light (~110 μmol quanta s^{-1} m^{-2}, photosynthetically active radiation ranging from 400 to 700 nm) and temperature (27 °C) controlled system. The variations in light intensity (light duration) and temperature were shown to be less than 3% relative to the standard deviation based on the continual record of a Hobo Pro Logger (Onset Computer Corp., Bourne, MA, USA), which collected data every 10 min. The dissolved oxygen (DO), salinity and pH of the test seawater in each glass bottle were measured using a HACH HQ30d Multimeter (Hach company, CO, USA) at the beginning, middle and end of the experiment. The results of the water quality parameters were as follows (mean ± standard deviation): 6.34 ± 0.21 mg/L DO, 36.6 ± 0.2 g/L salinity, and 8.09 ± 0.18 pH.

2.2. Determination of Zn^{2+} levels released from nZnOs

To estimate the Zn^{2+} levels released from nZnOs, the test solutions (0, 50, 100 and 200 μg/L) were triple prepared using filtered seawater (pH = 8.19, salinity = 35.8 g/L) as mentioned above and were gently aerated at the temperature ranged 24.1–24.8 °C for 24 h. The test solution then was filtered through a centrifugal filter (Amicon Ultra 10 k device, Millipore), corresponding to 2 nm cutoff for the size of nanoparticles (Trevisan et al., 2014). The filtrate was analyzed for the Zn^{2+} concentration using flame atomic absorption spectrometry (Model Z-5000, Hitachi) following APDC chelation and MIBK extraction procedures (APHA, 1992).

2.3. Examining the coral using scanning electron microscopy

After the nZnOs exposure, the coral fractions were fixed using 2.5% glutaraldehyde in seawater for 3 h followed by a dehydrating process in