Deep-Sea Research I 107 (2016) 59–69

Contents lists available at ScienceDirect

Deep-Sea Research I

journal homepage: www.elsevier.com/locate/dsri

Measurement of dark, particle-generated superoxide and hydrogen peroxide production and decay in the subtropical and temperate North Pacific Ocean

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ARTICLE INFO

Article history:
Received 15 November 2014
Received in revised form
24 October 2015
Accepted 25 October 2015
Available online 3 November 2015

Keywords:
Superoxide
Hydrogen peroxide
Reactive oxygen species
Chemiluminescence

ABSTRACT

Reactive oxygen species (ROS), which include the superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2), are thought to be generated mostly through photochemical reactions and biological activity in seawater and can influence trace metal speciation in the ocean. This study reports the results of an intercomparison of two methods to measure particle-generated [O_2^-] in seawater samples, as well as measurements of particle-generated O_2^- and H_2O_2 concentrations, decay kinetics, and dark production rates in seawater samples at Station ALOHA and (O_2^- only) in the southern California Current Ecosystem. O_2^- was measured using two different methods relying on chemiluminescence detection. The first method measured the difference between steady-state [O_2^-] in filtered and unfiltered seawater, while the second method (standard method) measured O_2^- decay to baseline in freshly filtered seawater. Because both methods detected [O_2^-] relative to the background signal from filtered seawater, both should have measured [O_2^-] generated by particles (presumably biota). However, the O_2^- concentrations determined by the first method were always much smaller than those obtained from the second (standard) method. Follow-up laboratory and field experiments showed that the increased signal in the standard method was due to a filtration artifact that could neither be eliminated nor consistently accounted for under the tested conditions. We therefore recommend the first method for measuring particle-generated [O_2^-]. Measured by this method, Station ALOHA had particle-generated O_2^- concentrations that ranged from undetectable to 0.02 nM, with production rates less than 0.6 nM hr^{-1} and decay rate coefficients from 0.003 to 0.014 s^{-1}. The southern California Current Ecosystem had particle-generated O_2^- concentrations that ranged from undetectable to 0.05 nM, with production rates up to 4.7 nM hr^{-1} and decay rate coefficients from 0.006 to 0.017 s^{-1}. H_2O_2 concentrations were measured by chemiluminescence detection, using dark incubations of unfiltered water samples to simultaneously determine production and decay rates. H_2O_2 concentrations at Station ALOHA ranged from 7 to 88 nM. Dark production rates and decay rate coefficients were low (mostly < 1.5 nM hr^{-1} and < 0.03 h^{-1}, respectively); higher values were detected when biota were pre-concentrated with net tows. These rates of ROS production are lower than those reported by previous studies in other regions of the Pacific Ocean, but could still be significant compared to photochemical production.

1. Introduction

The reactive oxygen species (ROS) superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are of interest in marine waters because their reactions with trace metals can alter metal speciation and therefore impact metal bioavailability. In addition, H_2O_2 is toxic to the cyanobacterium *Prochlorococcus* at environmentally relevant concentrations (Morris et al., 2011), raising the possibility of an ecological role of H_2O_2 in some parts of the ocean.

Until recently, the detection and measurement of ROS in the ocean has primarily focused on H_2O_2, which, with typical half-lives on the order of hours to days, is much more stable than O_2^-, whose half-life in seawater ranges from seconds to minutes (Hansard et al., 2010; Heller and Croot, 2010c; Rose et al., 2010, 2008b). Removal of O_2^- from the ocean occurs via multiple pathways. One removal pathway for O_2^- is by a disproportionation reaction to form H_2O_2 and O_2 (Cooper and Zika, 1983; Moffett and Zafiriou, 1990; Petasne and Zika, 1987). Redox reactions with trace metals and a redox cycling component of natural organic matter also occur (Goldstone and Voelker, 2000; Voelker and Sedlak, 1995; Voelker

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http://dx.doi.org/10.1016/j.dsr.2015.10.012
0967-0637/

et al., 2000; Zafiriou et al., 1998), which may catalyze O_2^- disproportionation while maintaining a fraction of the metal or organic compound in an unstable oxidation state.

In the ocean, O_2^- and H_2O_2 can be produced by photochemical reactions and by biological processes. Photochemically produced O_2^- and H_2O_2 are attributed to the photo-oxidation of chromophoric dissolved organic matter (CDOM) (Micinski et al., 1993). The photochemical production rate of O_2^- and H_2O_2 is influenced by the concentrations (Fan, 2008) and characteristics (marine vs. terrestrial) of the CDOM (Micinski et al., 1993). Numerous marine phytoplankton species as well as a variety of heterotrophic bacteria have been observed to produce extracellular O_2^- and H_2O_2 under laboratory conditions, even in the absence of light (e.g. Diaz et al. (2013); Kustka et al. (2005); Marshall et al. (2005); Rose et al. (2008b)). The ROS production rates observed in some culture studies (e.g. Diaz et al. (2013); Rose et al. (2008b)) appear to be sufficiently fast to contribute significantly to dark O_2^- and H_2O_2 production rates in some areas of the ocean.

With the recent development of a sensitive chemiluminescence flow-injection system to measure O_2^- in the ocean (Rose et al., 2008a), several studies have attempted to quantify the magnitude of biological O_2^- production by measuring $[O_2^-]$ versus time in freshly filtered seawater samples (Gulf of Alaska: Hansard et al., 2010; Costa Rica Dome upwelling region: Rose et al., 2008b; and the Great Barrier Reef lagoon: Rose et al., 2010). Because sampling occurred at night, or the sample was kept in the dark sufficiently long to eliminate any photochemical contribution to $[O_2^-]$, the $[O_2^-]$ extrapolated backwards to time of filtering was interpreted as the steady-state $[O_2^-]$ signal resulting from the balance of decay and dark, particle-generated (presumably biological) production, allowing calculation of a dark production rate. These studies concluded that significant dark production of O_2^- occurs in the regions that were studied.

Dark H_2O_2 production rates were measured concurrently with O_2^- production rates in only one of these studies, and both rates were of similar magnitude (Hansard et al., 2010). A 2:1 ratio between O_2^- and H_2O_2 production rates is the expected result if O_2^- forms H_2O_2 by disproportionation and if direct biological formation of H_2O_2 is not significant. However, O_2^- does not necessarily form H_2O_2 in the expected ratio. Rose et al. (2010), in their study at the Great Barrier Reef, observed a significant increase in H_2O_2 production rates when superoxide dismutase was added to ensure disproportionation, indicating that in the absence of the added enzyme, the majority of the O_2^- did not form H_2O_2. In such cases, production rates of O_2^- may be greater than those of H_2O_2. However, the opposite may also occur, since direct biological production of H_2O_2, without O_2^- as a precursor, is also possible (Kim et al., 2007; Palenik et al., 1987). Thus, concurrent measurements of O_2^- and H_2O_2 production rates can provide information on the source of H_2O_2 and the reactions of O_2^- in a water sample.

The main goal of this study was to measure particle-generated O_2^- concentrations and decay rates at Station ALOHA and in the southern California Current Ecosystem, in order to quantify the dark (presumably biological) production rate of O_2^- in these regions. Station ALOHA was the first subtropical, *Prochlorococcus*-dominated region to be examined using these techniques, and the southern California Current ecosystem provided a comparison to a less oligotrophic region. O_2^- measurements were made using two different chemiluminescence flow injection methods, a new method comparing steady-state $[O_2^-]$ in unfiltered and filtered seawater, and the standard method of observation of O_2^- in freshly filtered seawater and extrapolation to time of filtering (Hansard et al., 2010; Heller and Croot, 2010c; Rose et al., 2008b, 2010). The results and potential difficulties of measuring O_2^- by these two different methods are discussed. A secondary goal of the study was to measure H_2O_2 concentrations, production and decay rates, and to compare H_2O_2 dark production rates to those measured for O_2^-.

2. Materials and methods

2.1. Seawater collection

All seawater was collected at Station ALOHA from July 10 to 25, 2012 or in the southern California Current Ecosystem from April 9 to 11, 2013. Three stations were occupied in the California Current; Station 1 (32°57'4.98″N, 117°40'26.28″W), Station 2 (34°19'40.68″N, 120°49'0.60″W) and Station 3 (33°43'35.64″N, 119°14'28.68″W). Seawater was collected with 5 L GO-Flo bottles (General Oceanics) attached to a kevlar line. The Station ALOHA casts were conducted ~1 h before the 0800 CTD cast. The California Current casts took place immediately after the CTD casts, which were 1200–1500 (cast 1 and 2) or 0800 (cast 3). Once the GO-Flo bottles were retrieved, analysis for O_2^- in the samples began 30 min (deep Station ALOHA samples) to 8 h after collection. Samples were in the dark for at least 10 half lives prior to analysis and were kept in the dark during analysis to ensure that photochemically produced O_2^- did not contribute to the measured values. Depth profiles at Station ALOHA were analyzed from deep to surface samples except on July 24 and 25, when samples were analyzed from shallow to deep. The depth profiles usually consisted of samples from 25 m, 75 m and the deep-chlorophyll-maximum (105–130 m). On alternate days, only a 25 m water sample was collected for analysis. The California Current samples were kept in the dark in a room temperature (~17 °C) water bath for a minimum of 30 min prior to analysis to minimize any effects of temperature changes during analysis (Hansard et al., 2010). The California Current profiles were analyzed from surface to deep and consisted of surface (20–25 m) and deeper samples (40–50 m). Seawater was filtered by slightly pressurizing (< 7 PSI) the GO-Flo bottle or a 500 mL dark Teflon bottle (California Current) with filtered (0.2 μm PTFE filters, Whatman) compressed air into a 125 mL dark HDPE bottle. The cartridge filter (0.2 μm Supor cartridge, PALL) was acid washed with 0.1 M trace metal grade HCl, rinsed with Milli-Q and stored in Milli-Q until use. Prior to collection of filtered seawater for analysis, 1 L of seawater was passed through the cartridge filter. At Station ALOHA, the same filter was used for all depths for ~10 days before using a new filter. For the California Current samples, a new cartridge filter was used for each sample.

2.2. Measurement of O_2^- in natural water

O_2^- was detected with a flow injection system (FeLume Mini, Waterville Analytical) with the use of the MCLA (2-methyl-6-[*p*-methoxyphenyl]-3,7-dihydtoimidazo[1,2-*a*]pyrazin-3-one) chemiluminescence probe. The flow injection system was set up to directly pump the seawater sample and MCLA reagent into a homemade flow cell, made of tygon tubing, which sat directly under a photomultiplier tube (PMT, Hamamatsu). The PMT was set to integrate the signal over 20 ms, a collection time of 10 periods, with the default setting for PMT voltage, and two data points were collected every second, except for Cast 3 in the California Current where the integration time was increased to 50 ms. Two different PMTs were used on the cruises, model H9319-11 at Station ALOHA and model HC135-11 in the California Current. The seawater sample and MCLA reagent were each pumped at a flow rate of 3 ml min^{-1}. At this rate it took ~20 s to pump fresh reagent and sample to the flow cell. The MCLA reagent was composed of 30 μM diethylenetriaminepentaacetic acid (DTPA) (Sigma), 5 μM MCLA (TCI America), and 0.2 M MES hydrate buffer (Sigma), adjusted to pH 6.0 with ~0.074 M NaOH (Sigma). All measurements were