Available online at www.sciencedirect.com

ScienceDirect

Geochimica et Cosmochimica Acta 232 (2018) 244–264

Geochimica et Cosmochimica Acta

www.elsevier.com/locate/gca

# The molecular products and biogeochemical significance of lipid photooxidation in West Antarctic surface waters

James R. Collins [a,b,*], Helen F. Fredricks [a], Jeff S. Bowman [c,1], Collin P. Ward [a], Carly Moreno [d], Krista Longnecker [a], Adrian Marchetti [d], Colleen M. Hansel [a], Hugh W. Ducklow [c], Benjamin A.S. Van Mooy [a,*]

[a] Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA
[b] MIT/WHOI Joint Program in Oceanography/Applied Ocean Science and Engineering, Woods Hole, MA 02543, USA
[c] Division of Biology and Paleo Environment, Lamont-Doherty Earth Observatory, Columbia University, Palisades, NY 10964, USA
[d] Department of Marine Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Received 6 February 2018; accepted in revised form 27 April 2018; available online 7 May 2018

## Abstract

The seasonal depletion of stratospheric ozone over the Southern Hemisphere allows abnormally high doses of ultraviolet radiation (UVR) to reach surface waters of the West Antarctic Peninsula (WAP) in the austral spring, creating a natural laboratory for the study of lipid photooxidation in the shallow mixed layer of the marginal ice zone. The photooxidation of lipids under such conditions has been identified as a significant source of stress to microorganisms and short-chain fatty acids altered by photochemical processes have been found in both marine aerosols and sinking marine particle material. However, the biogeochemical impact of lipid photooxidation has not been quantitatively compared at ecosystem scale to the many other biological and abiotic processes that can transform particulate organic matter in the surface ocean. We combined results from field experiments with diverse environmental data, including high-resolution, accurate-mass HPLC-ESI-MS analysis of lipid extracts and *in situ* measurements of ultraviolet irradiance, to address several unresolved questions about lipid photooxidation in the marine environment. In our experiments, we used liposomes—nonliving, cell-like aggregations of lipids—to examine the photolability of various moieties of the intact polar diacylglycerol (IP-DAG) phosphatidylcholine (PC), a structural component of membranes in a broad range of microorganisms. We observed significant rates of photooxidation only when the molecule contained the polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA). As the DHA-containing lipid was oxidized, we observed the steady ingrowth of a diversity of oxylipins and oxidized IP-DAG; our results suggest both the intact IP-DAG the degradation products were amenable to heterotrophic assimilation. To complement our experiments, we used an

*Abbreviations: AQY, apparent quantum yield; BHT, butylated hydroxytoluene; DCM, dichloromethane; DGCC, diacylglyceryl carboxyhydroxymethylcholine; DGDG, digalactosyldiacylglycerol; DGTA, diacylglyceryl hydroxymethyl-trimethyl-β-alanine; DGTS, diacylglyceryl trimethylhomoserine; DHA, docosahexaenoic acid; DNP-PE, dinitrophenyl-phosphatidylethanolamine; ESI, electrospray ionization; FFA, free fatty acid; FSFA, fully saturated fatty acid; GlyPCho, glycerophosphocholine; HAc, acetic acid; HPLC, high-performance liquid chromatography; IP-DAG, intact polar diacylglycerol; IPL, intact polar lipid; LPC, lysophosphatidycholine; MeOH, methanol; MDA, malondialdehyde; MGDG, monogalactosyldiacylglycerol; MUFA, monounsaturated fatty acid; DUFA, diunsaturated fatty acid; Ox-IPL, oxidized intact polar lipid; Ox-PC, oxidized phosphatidylcholine; PC, phosphatidylcholine; PCho, phosphocholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PTFE, polytetrafluoroethylene; PUFA, polyunsaturated fatty acid; SQDG, sulfoquinovosyldiacylglycerol; TAG, triacylglycerol; TBA, thiobarbituric acid; Tris, tris(hydroxymethyl)aminomethane; UVA, ultraviolet-A (315-400 nm); UVB, ultraviolet-B (290-315 nm); UVR, ultraviolet radiation*

* Corresponding authors at: School of Oceanography and eScience Institute, University of Washington, Seattle, WA 98195, USA (J.R. Collins).

*E-mail addresses:* james.r.collins@aya.yale.edu (J.R. Collins), bvanmooy@whoi.edu (B.A.S. Van Mooy).

[1] Present address: Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093, USA.

https://doi.org/10.1016/j.gca.2018.04.030

enhanced version of a new lipidomics discovery software package to identify the lipids in water column samples and in several diatom isolates. The galactolipid digalactosyldiacylglycerol (DGDG), the sulfolipid sulfoquinovosyldiacylglycerol (SQDG) and the phospholipids PC and phosphatidylglycerol (PG) accounted for the majority of IP-DAG in the water column particulate (≥0.2 μm) size fraction; between 3.4 and 5.3% of the IP-DAG contained fatty acids that were both highly polyunsaturated (i.e., each containing ≥5 double bonds). Using a broadband apparent quantum yield (AQY) that accounted for direct and Type I (i.e., radical-mediated) photooxidation of PUFA-containing IP-DAG, we estimated that $0.7 \pm 0.2$ μmol IP-DAG $m^{-2}$ $d^{-1}$ ($0.5 \pm 0.1$ mg C $m^{-2}$ $d^{-1}$) were oxidized by photochemical processes in the mixed layer. This rate represented 4.4% (range, 3–21%) of the mean bacterial production rate measured in the same waters immediately following the retreat of the sea ice. Because our liposome experiments were not designed to account for oxidation by Type II photosensitized processes that often dominate in marine phytodetritus, our rate estimates may represent a sizeable underestimate of the true rate of lipid photooxidation in the water column. While production of such diverse oxidized lipids and oxylipins has been previously observed in terrestrial plants and mammals in response to biological stressors such as disease, we show here that a similar suite of molecules can be produced via an abiotic process in the environment and that the effect can be commensurate in magnitude with other ecosystem-scale biogeochemical processes.


*Keywords:* Lipid photooxidation; Oxidized lipids; Oxylipins; Intact polar diacylglycerolipids; Phosphatidylcholine; Apparent quantum yield; Lipidomics; West Antarctica; Ultraviolet radiation; Polyunsaturated fatty acids

## 1. INTRODUCTION

The seasonal depletion of stratospheric ozone over the Southern Hemisphere continues to allow abnormally high doses of ultraviolet radiation (UVR) to reach the land and ocean surface in much of Antarctica (IPCC, 2005; Laube et al., 2014). The negative effects of UVR on marine plankton are mediated primarily via reactive oxygen species (ROS) and include shifts in bulk cellular lipid composition, reduced cell growth rates, direct damage to critical biochemicals such as DNA and proteins (Worrest, 1983; Moreau et al., 2016), and cell mortality (Davidson et al., 1994; Davidson and Marchant, 1994; Karentz, 1994; Neale et al., 1994; Prézelin et al., 1994; Vernet et al., 1994; Helbling et al., 1996; Hessen et al., 1997; Skerratt et al., 1998; Mock and Kroon, 2002). In the West Antarctic Peninsula (WAP) specifically, UVR exposure has been correlated with declines in marine primary production (Schofield et al., 1995).

ROS can also oxidize and damage acyl-containing lipids such as intact polar diacylglycerols (IP-DAG), the primary structural components of many cell and organelle membranes (Murphy, 1983; Crastes de Paulet et al., 1988; Kramer et al., 1991). Diatoms and other phytoplankton that inhabit high-latitude waters such as those of the WAP may contain as much as 30% lipid; this lipidome is often dominated by IP-DAG containing polyunsaturated fatty acids (PUFA, i.e., those containing ≥2 double bonds; Palmisano et al., 1988; Nichols et al., 1989; Skerratt et al., 1998), which are particularly susceptible to peroxidation (Girotti, 1990, 1998; Wagner et al., 1994). Laboratory studies and experiments in other environmental systems have shown that peroxidation of PUFA can produce hundreds of bioactive lipid derivatives, collectively termed oxylipins. Oxylipins of enzymatic origin are known to play critical roles as intercellular signals, stress mediators, and predator defense mechanisms in various diatoms isolated from mid-latitude ecosystems (Miralto et al., 1999; Wichard et al., 2005; Barofsky and Pohnert, 2007; Fontana et al., 2007; Leflaive and Ten-Hage, 2009; Lauritano et al., 2011, 2012); these molecules can also affect growth rates of marine heterotrophic bacteria (Ribalet et al., 2008) and regulate metabolism of bacteria associated with sinking particles (Edwards et al., 2015). Various diatoms typically dominate sea-ice and ice-edge communities during early stages of blooms in Antarctic waters (Lizotte, 2001).

While the biological production and bioactivity of diatom-derived oxylipins has received significant scientific attention in oceanography and UVR-induced oxylipin production has been characterized in higher plants and other organisms (Girotti, 1990, 1998; Halliwell and Chirico, 1993), the non-enzymatic generation of oxylipins and other oxidized lipid derivatives in the environment has received comparatively little scientific attention. Neither the biological nor abiotic production of larger oxidized lipid products such as intact oxidized polar lipids (ox-IPL; e.g., Buseman et al., 2006; Domingues et al., 2008; O'Donnell, 2011; Vu et al., 2012; Spickett and Pitt, 2015) has been investigated in the ocean or other natural environment. Rontani and others (Marchand and Rontani, 2001; Rontani, 1999, 2001; Christodoulou et al., 2010; Rontani et al., 2016, 2012a, 2012b, 1998) have established that Type II (i.e., involving singlet oxygen) photooxidation of mono- and di-unsaturated fatty acids in phytodetritus is a process significant enough to be detected via short-chain oxylipin biomarkers in sinking marine particulate material, but the photooxidation of intact polar lipids in high-latitude marine microbial biomass has not been directly investigated. In addition, while short-chain fatty acids altered by photochemical processes have been used to estimate the photooxidation state of organic matter in sinking marine particles in the Arctic (Rontani, 1999; Rontani et al., 2012a; Amiraux et al., 2017) and carboxylic acids of photochemical origin have been previously identified in marine aerosols (Kawamura and Gagosian, 1987, 1990), few estimates exist of the relative biogeochemical impact of lipid photooxidation in the surface ocean compared to the many other biological and abiotic processes that can transform particulate organic matter.