Organic Geochemistry 77 (2014) 59–71

Contents lists available at ScienceDirect

Organic Geochemistry

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

Sequential photo- and autoxidation of diatom lipids in Arctic sea ice

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ARTICLE INFO

Article history:
Received 4 March 2014
Received in revised form 1 August 2014
Accepted 24 September 2014
Available online 5 October 2014

Keywords:
Arctic
Sea ice diatoms
HBI alkenes
Lipids
Autoxidation
Photooxidation

ABSTRACT

We measured the concentrations of selected lipids and some of their degradation products in a time series of sea ice samples collected from Resolute Bay in the Canadian Arctic in 2012. The identification of specific tracers of photo- and autoxidation reactions provided evidence of both abiotic processes acting on organic matter, although none of the lipids appeared sensitive to significant biodegradation. Some differences in lipid reactivity were observed between the upper (3–10 cm) and lower (0–3 cm) sections of the cores, possibly as a result of increased exchange at the ice–water boundary for the latter. In terms of photodegradation, the phytyl side chain of chlorophyll and a tri-unsaturated highly branched isoprenoid (HBI) alkene were most affected, 24-methylenecholesterol (from diatoms) was more susceptible to photodegradation than brassicasterol (from diatoms and prymnesiophytes), while sitosterol and cholesterol, likely from a range of sources, were largely unaffected. With respect to autoxidation, the reactivity trend for the sterols was reversed, with significant autoxidation of sitosterol and cholesterol, but not of 24-methylenecholesterol or brassicasterol. The phytyl side chain of chlorophyll and a highly branched isoprenoid triene were also particularly susceptible to autoxidation. In contrast, the diatom fatty acid, $C_{16:1}$, was not substantially altered by any degradation pathway. By measuring temporal changes in the proportions of specific tracers of each degradation process, we provide evidence that such abiotic reactions take place via sequential photooxidation and autoxidation, with homolytic cleavage of photochemically produced hydroperoxides leading to autoxidation of initial substrates and subsequent oxidation products. The observation of significant abiotic degradation of several lipids appears to take place despite the low temperature in sea ice (ca. 0 °C) and should be considered carefully alongside other removal processes involving organic matter in marine systems.

1. Introduction

The fate of marine organic matter (OM) involves a range of biotic and abiotic degradation processes which can be especially important for determining the nature and extent of removal of phytodetritus both prior to, and following, burial in the sedimentary environment (Wakeham and Lee, 1989; Henrich, 1992; Wakeham and Canuel, 2006). Abiotic degradation processes have received less attention than their biologically-mediated (heterotrophic) counterparts and fall into two main categories: photooxidation and autoxidation (free radical degradation).

Lipid photooxidation can be intense during the senescence of phytoplankton in the euphotic layer of the oceans due to the presence of chlorophyll, which is a very efficient photosensitizer (Foote, 1976) capable of generating singlet oxygen (Type II photoprocesses). When chlorophyll absorbs a quantum of light energy, an excited singlet state (^{1}Chl) is formed which, in healthy cells, leads predominantly to the characteristic fast reactions of photosynthesis (Foote, 1976). However, a small proportion of ^{1}Chl undergoes intersystem crossing (ISC) to form the longer lived triplet state (^{3}Chl; Knox and Dodge, 1985). ^{3}Chl can not only take part in Type I reactions (H atom or electron abstraction) (Knox and Dodge, 1985), but it can also generate highly reactive oxygen species (ROS) and, in particular, singlet oxygen ($^1O_2$), by reaction with ground state oxygen ($^3O_2$) via Type II processes. Since the fast reactions of photosynthesis do not operate in senescent phototrophic organisms, an accelerated rate of formation of ^{3}Chl and $^1O_2$ would be expected (Nelson, 1993). Indeed, photochemically generated $^1O_2$ plays a key role in the degradation of unsaturated lipid components within senescent phytoplanktonic cells, with production of allylic hydroperoxides (for a review see Rontani, 2012) that undergo a range

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http://dx.doi.org/10.1016/j.orggeochem.2014.09.009
0146-6380/

of further reactions, leading to overall degradation. Amongst these, the metal ion-catalyzed homolytic cleavage (Schaich, 2005) of photochemically-produced hydroperoxides has been suggested to play an important role in the initiation of autoxidation reactions in phytodetritus (Rontani et al., 2003), although, this has not been firmly established experimentally. In any case, the resulting alkoxyl and peroxyl radicals are highly reactive towards C=C bonds and other reactive sub-structures (Fossey et al., 1995) in OM, resulting in autoxidative degradation. In contrast to photooxidation, however, autoxidation is not confined to the euphotic zone and can occur throughout the water column and in sediments.

A number of recent studies have demonstrated the importance of abiotic processes acting on OM in sinking (Christodoulou et al., 2009; Rontani et al., 2012a) and suspended (Rontani et al., 2009, 2011) particles, together with that deposited in surface sediments (Rontani et al., 2012b). In addition, the reactive species generated as a result of photochemical sensitization (e.g. $^1O_2$) can also impact on bacterial communities (Rontani et al., 2003; Christodoulou et al., 2010), thereby limiting their associated degradation pathways, and on faecal zooplankton material (Rontani et al., 2012a).

It has also been demonstrated that the efficiency of direct photochemical processes (e.g. chlorophyll photodegradation) decreases with temperature (SooHoo and Kiefer, 1983). The efficiency of Type II photosensitized processes in senescent phytoplanktonic cells seems to be limited mainly by two factors: (i) the diffusion rate of $^1O_2$ outside biological membranes and (ii) the photodegradation of the sensitizer (chlorophyll). Decreases in the diffusion rate of $^1O_2$ (Ehrenberg et al., 1998) and in the rate of photodegradation of chlorophyll (SooHoo and Kiefer, 1983), generally observed at low temperature, should thus favour photosensitizing effects in Arctic phytodetritus. This assumption is in agreement with some of our recent observations (Rontani et al., 2012a,b).

The aim of the current study was twofold: first, to investigate the photodegradation of phytodetritus at low temperature (ca. 0 °C) and second, to see if the proposed evolution of the photochemical and autoxidation routes could be demonstrated through analysis of samples within a time series (not readily achieved within sediments, at least, or other sample types where there is time averaging of composition). To achieve these aims, we analyzed a time series of sea ice samples from the Canadian Arctic and, in particular, identified and (in most cases) quantified, fatty acids (FAs), selected sterols and highly branched isoprenoid (HBI) alkenes. To test for degradation reactions, we also sought specific tracers of photooxidation and autoxidation for each lipid identified previously (for a review see Rontani, 2012).

2. Experimental

2.1. Sea ice samples

A time series of sea ice samples was collected from 19 May to 12 June 2012 from Resolute Passage (74.73°N; 95.56°W) in the Canadian Arctic under the umbrella of the Arctic-ICE (ice covered ecosystem) project as described by Belt et al. (2013) (Table 1). Ice cores were obtained using a 9 cm internal diameter Mark II coring system (Kovacs Enterprises) and sectioned in the field. Pooled ice core sections (×3) corresponding to the 0–3 cm (A samples) and 3–10 cm (B samples) sections above the ice–ocean interface were allowed to melt in filtered seawater (FSW; 0.2 μm) using at least 2 parts FSW to 1 part ice melt, filtered (Whatman GF/F) and stored frozen (−20 °C or −80 °C) prior to analysis.

2.2. Sample treatment

OM recovered on filters was reduced with excess $NaBH_4$ in MeOH (25 ml; 30 min) to reduce labile hydroperoxides (resulting from photo- or autoxidation) to alcohols which are more amenable to analysis using gas chromatography–electron ionization mass spectrometry (GC–EIMS; see below). Water (25 ml) and KOH (2.8 g) were then added and the resulting mixture saponified by refluxing (2 h). After cooling, the mixture was acidified (HCl, 2 N) to pH 1 and extracted with dichloromethane (DCM; 3 × 20 ml). The combined DCM extracts were dried over anhydrous Na_2SO_4, filtered and concentrated via rotary evaporation at 40 °C. Since HBI oxidation product content was quite low relative to other lipids, accurate quantification required further separation of the extract, which was therefore fractionated using column chromatography (silica; Kieselgel 60, 8 × 0.5 cm). HBI alkenes were obtained by elution with hexane (10 ml) and their oxidation products by subsequent elution with toluene (10 ml). Additional elution with MeOH (10 ml) was carried out to recover the more polar lipid compounds.

2.3. Derivatization

For all samples containing hydroxylic components, an aliquot was dissolved in 300 μl pyridine/bis(trimethylsilyl)trifluoroacetamide (BSTFA, Supelco; 2:1, v:v) and silylated (1 h) at 50 °C. After evaporation to dryness under a stream of N_2, the derivatized residue was dissolved in a mixture of EtOAc and BSTFA (to avoid desilylation) and analyzed via GC–EIMS.

2.4. Assignment and quantification of lipids and their degradation products

Compounds were assigned by comparison of retention times and mass spectra with those of standards and quantified (calibration with external standards) using GC–MS in EI mode. For low concentrations, or in the case of co-elution, quantification was achieved using single ion monitoring (SIM) as described by Rontani et al. (2009). GC–MS was carried out with an Agilent 6890 gas chromatograph connected to an Agilent 5973 Inert mass spectrometer. GC conditions were: 30 m × 0.25 mm (i.d.) fused silica column coated with R-Xi®-5MS (Restek; 0.25 μm film thickness); oven programmed in three sequential steps: (i) 70–130 °C at 20 °C/min, (ii) to 250 °C at 5 °C/min and (iii) to 300 °C at 3 °C/min; carrier gas (He) at 0.69 bar until the end of the temperature programme and then programmed from 0.69 bar to 1.49 bar at 0.04 bar/min; injector (on column with retention gap) 50 °C; electron energy 70 eV; source temperature 190 °C; cycle time 1.99 and 8.3 cycles/sin scan and SIM (single ion monitoring) modes, respectively.

2.5. Quantification of HBI degradation products

HBI oxidation products were quantified using an Agilent 7850-A gas chromatograph connected to an Agilent 7000-QQQ mass spectrometer. The following conditions were employed: 30 m × 0.25 mm (i.d.) fused silica column coated with HP-5MS (Agilent; film thickness: 0.25 μm); oven programmed from 70 to 130 °C at 20 °C/min, then to 250 °C at 5 °C/min and then to 300 °C at 3 °C/min; carrier gas (He), 1.0 bar; injector (splitless), 250 °C; electron energy, 70 eV; source temperature, 230 °C; quadrupole temperature, 150 °C; scan range m/z 40–700; collision energy, ranging from 5 to 15 eV; collision flow, 1.5 ml/min (N_2); quench flow, 2.25 ml/min (He); cycle time, 0.2 s. Oxidation products were assigned by comparison of retention times and mass spectra with those of standards. Quantification of HBI oxidation products was carried out with external standards in MRM (multiple reaction monitoring) mode. Precursor ions were selected from the more intense ions (and specific fragmentations) observed in EI spectra. More efficient and specific MRM transitions were selected