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Effects of growth stages, respiration, and microbial degradation of phytoplankton on cellular lipids and their compound-specific stable carbon isotopic compositions

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ABSTRACT

A series of laboratory experiments were conducted to examine effects of biochemical processes on phytoplankton lipids and their stable carbon isotopic compositions. *Thalassiosira weissflogii* and *Emiliania huxleyi* were axenically cultivated in batch cultures through exponential growth (phase I) and stationary period (phase II), followed by dark respiration (phase III) and subsequent microbial degradation (phase IV). The results indicated that the bulk parameters, lipid contents and associated isotopic compositions of two phytoplankton species varied differently during different cell growth stages, cell respiration and microbial degradation processes. *T. weissflogii* continuously produced neutral lipids and fatty acids at similar rates between phase I and phase II. By contrast, *E. huxleyi* produced neutral lipids at faster rates in phase II than in phase I although the production rates of fatty acids were still similar between the two phases. The lipid isotopic compositions of these two phytoplankton species varied in a similar pattern through cell growth: remarkably positive shift in phase I but relatively small negative shift or little change in phase II. During cell respiration (phase III), two phytoplanktons lost their lipids by varying percentages but the isotopic compositions of all compounds remained relatively constant. With degradation of lipids in phase IV, their isotopic compositions varied in three different ways: positive shift for most fatty acids; little change for sterols and alkenones; and a negative shift for 20:5 fatty acid of *T. weissflogii*. This experimental study implies: (1) isotopic compositions of phytoplankton lipids are heterogeneously generated through different cell growth phases, likely dependent on cell physiological states, medium conditions, and relative proportions of intracellular components; and (2) microbial degradation plays a more important role in altering isotopic compositions of lipid compounds than cell respiration, probably related to different labilities of lipids between different cellular components.

1. Introduction

Marine phytoplankton produce approximately 5×10^{16} g organic carbon each year in oceans (Martin et al., 1987), and most phytoplankton-produced organic compounds are recycled in the water column and surface sediments through biological and biochemical processes (Harvey et al., 1995). Phytoplankton lipids account for a relatively smaller fraction (~5–20%) of total organic matter in phytoplankton biomass (Parsons et al., 1961), but some specific lipid compounds such as sterols, alkenones, and fatty acids have been widely used as biomarkers to study food web dynamics (Kainz et al., 2004; Shin et al., 2008), organic carbon cycling (Volkman et al., 1998; Wakeham et al., 1997) and paleoceanographic records (Bac et al., 2003; Müller et al., 1998; Prahl and Wakeham, 1987) because their structures are more source-specific and less reactive than proteins and carbohydrates (Harvey et al., 1995). Furthermore, the compound-specific stable isotopic compositions of lipid biomarkers have been developed as a analytical tool to characterize the microbial community (Boschker and Middelburg, 2002) and reconstruct ancient atmospheric pCO_2 records (Jasper and Hayes, 1994; Pagani et al., 1999, 2002).

Previous studies have suggested that the chemical and isotopic compositions of algal lipid biomarkers are largely influenced by environmental conditions such as light and UV-B irradiation (Brown et al., 1996; Skerratt et al., 1998), temperature and salinity (Xu and Beardall, 1997; Zhu et al., 1997), and concentrations of nutrient and CO_2 (Dempster and Sommerfield, 1998; Riebesell et al., 2000). Cellular lipids are also dependent on cell physiological states or cell growth phase (Dunstan et al., 1993; Lv et al., 2010; Mansour et al., 2003). Moreover, the isotopic compositions of algal lipids are controlled by carbon acquisition mechanisms (Laws et al., 1998; Rost et al., 2002), compound synthesis pathways (Laws et al., 2001), algal growth rate (Benthien et al., 2007; Laws et al., 1995; Popp et al., 1998a), and cell size and geometry (Burkhardt et al., 1999; Popp et al., 1998b). However, few systematic studies have been conducted to examine how individual lipids and their isotopic compositions of

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different phytoplankton species vary through different growth phases.

Marine phytoplankton biosynthesize various lipids for different functions in different intracellular structures over different growth stages (Mansour et al., 2003; Sukenik and Carmeli, 1990). Most species produce polar phospholipids in their cell membrane as structural components but they also make different lipids as energy storage compounds in their cytoplasm (Bell and Pond, 1996; Brown et al., 1996). It has been observed that more structural lipids are produced during exponential growth phase (Mansour et al., 2003; Volkman et al., 1989) while more storage lipids are made by phytoplankton cells during stationary phase (Dunstan et al., 1993; Epstein et al., 2001; Lv et al., 2010).

It is thought that the carbon isotopic compositions of phytoplankton and individual compounds are mainly controlled by the availability of aqueous CO_2 (Arthur et al., 1985; Hayes et al., 1989; Rau et al., 1989). Laws et al. (1995) further pointed out that both CO_2 concentration and cell growth rate are major factors controlling the isotopic fractionation of carbon by phytoplankton. More evidence (Bidigare et al., 1997) suggested that variations in cell growth rate had a critical impact on the isotopic compositions of alkenones. However, the biosynthetic pathways and intracellular distributions of alkenones during cell growth under variable conditions are largely unknown (Laws et al., 2001). In addition, it has not been clarified whether isotopic compositions of lipid compounds vary in different cellular structures.

Cell respiration and microbial degradation are two important processes to recycle algal lipids in natural environments (Eltgroth et al., 2005; Manoharan et al., 1999). While cell respiration mainly consumes lipids bound in intracellular storage compartments (Brown et al., 1996; Manoharan et al., 1999), microbial degradation may decompose lipids in both membrane and intracellular storage compartments (Ding and Sun, 2005). So far, there have been few studies to directly distinguish the relative roles of respiration and microbial process in degrading algal lipids. Moreover, little is known about the effects of respiration and microbial degradation on the isotopic composition of phytoplankton-produced lipid compounds.

Applications of biomarkers and their molecular isotopic compositions are fundamentally based on a controversial assumption that biogeochemical cycling processes such as the degradation of organic matter have an insignificant impact on these signals after they are generated (Hayes et al., 1990; Pagani et al., 1999). Indeed, many studies (Freeman et al., 1994; Huang et al., 1997; Mazeas et al., 2002) showed that degradation of some organic compounds such as *n*-alkanes and polycyclic aromatic hydrocarbons did not result in significant changes in their isotopic compositions. However, other evidence has indicated that different organic compounds from various sources may experience diversified changes in their isotopic composition during degradation (Macko et al., 1994; Sun et al., 2004; Teece and Fogel, 2007). Nevertheless, what mechanisms cause these diversified isotopic alterations for different biomarkers during biogeochemical cycling processes has not been well understood.

This experimental study was designed to examine the effects of biological and biochemical processes on phytoplankton lipids and associated molecular isotopic compositions. Two main focuses are: (1) generation of lipids and their stable carbon isotopic compositions through different cell growth phases; and (2) relative roles of cell respiration (auto-metabolism) and microbial degradation in altering chemical and isotopic signals. *Thalassiosira weissflogii* (diatom) and *Emilliania huxleyi* (haptophyte) were axenically cultivated in batch cultures through exponential growth and stationary phase, followed by cell respiration, and then microbial degradation by introducing natural seawater. Bulk parameters (POC, TN, C/N, δ^{13}C-POC, and δ^{15}N-TN), lipids (sterols, alkenones, and fatty acids) and their isotopic compositions from experimental samples were analyzed to examine the influences of cellular physiological state and biochemical processes on phytoplankton lipids and their isotopic compositions.

2. Material and methods

2.1. Materials

Marine diatom *T. weissflogii* (CCMP1010) and marine haptophyte *E. huxleyi* (CCMP371) were obtained from the Provasoli–Guillard National Center for Culture of Marine Phytoplankton (CCMP). The strain of *T. weissflogii* was originally isolated from the Gulf Stream (37°N, 65°W) in December 1969 and the strain of *E. huxleyi* from the Sargasso Sea (32°N, 62°W; 50 m water depth) in June 1987. Two strains were made and remained axenic (confirmed by routine tests) in the CCMP. Seawater was collected from the Georgia coast (31°05′N, 81°16′W; in September 2005 and 31°25′N, 77°21′W; in May 2006) and from the Gulf of Mexico (28°16′N, 91°59′W; in September 2006 and 28°10′N, 91°36′W; in May 2008) by directly pumping surface (1 m) water into large carboys (50 L). The seawater used for medium preparation was filtered through Whatman 934-AH glass microfiber filters (pre-combusted at 450 °C for 4 h) and then sterilized by autoclaving. The f/2 (for *T. weissflogii*) and f/50 (for *E. huxleyi*) media were prepared according to the protocols recommended by the CCMP (Guillard, 1975; Guillard and Ryther, 1962). The seawater used for microbial degradation experiments was stored in carboys until incubation experiments start.

2.2. Experimental set up

First, *T. weissflogii* and *E. huxleyi* cells were axenically cultivated in batch cultures (duplicate for each species) in f/2 and f/50 media (~2.3 L) respectively under a light:dark (12 h:12 h) regime with a constant light intensity (50 μmol photons m^{-2} s^{-1}). The cultures were shaken twice by hand and cell densities in each culture were counted every day. Algal cells were cultured through exponential growth phase (phase I: 12 d for *T. weissflogii* and 10 d for *E. huxleyi*) and stationary phase (phase II: 14 d for *T. weissflogii* and 32 d for *E. huxleyi*). Then, the *T. weissflogii* cells were kept under continuous dark conditions over 14 d and *E. huxleyi* over 21 d (phase III). After that, the cultures were equally (100 mL) divided into a set of small flasks and frozen below −30 °C overnight to kill the cells. After thawing, 50 mL of natural seawater was added into each flask to introduce natural bacterial assembly. They were then incubated in the dark and aerobic conditions for 40 d by purging with air (phase IV).

2.3. Sampling and cell counting

Transfer and sampling of cultures were carried out in a sterile hood, which was pre-irradiated with a UV lamp for 30 min. Before sampling, the flasks were gently shaken by hand for a while to make uniform cell cultures. One milliliter of culture was taken every 1–2 d during phases I, II and III, and the cells were counted using an Olympus BH-2 microscope with a hemacytometer (Hausser Scientific). Subsamples (25–300 mL, depending on cell density) in all phases were collected at certain time points by filtering the cultures through glass microfiber filters (Whatman 934-AH) and then stored at −40 °C for future lipid extraction and analysis.

2.4. Analysis of bulk parameters

To monitor variations of bulk parameters (POC, TN, C/N, δ^{13}C-POC, and δ^{15}N-TN) of each alga, parallel experiments (phases I, II, and III) were conducted. Subsamples (15–200 mL, based on cell density) at certain time points were collected by filtering the cultures through glass microfiber filters (Whatman 934-AH, precombusted at 450 °C for 4 h) and kept frozen below −40 °C until analysis. Thawed samples were treated with 10% HCl to remove inorganic carbon, air-dried in a fume hood and then analyzed for the bulk parameters using a Carlo