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Nutrient temperature and light stress alter phosphorus and carbon forms in culture-grown algae

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ABSTRACT

Particulate organic matter (POM) in seawater contains a wide range of chemical compounds, many of which come directly from phytoplankton. Phytoplankton may alter their biochemical characteristics in response to stress, which in turn alters the relative abundance of carbon (C) and phosphorus (P) compounds in the POM. The objective of this research was to examine changes in the P and C forms of algae cultured under light intensity, temperature and P nutrition stress, using ^{13}C CPMAS and ^{31}P NMR spectroscopy. Of the C forms, lipids and protein were significantly related to light intensity, while lipids were significantly increased at high temperatures. Low-P nutrition significantly altered ^{13}C NMR spectra, but did not alter modeled C forms. There were few changes in P forms with light and temperature stress. Low- and high-P nutrition altered C:P and N:P ratios. Low-P nutrition did not alter P forms. High-P nutrition increased pyrophosphate, indicating luxury P consumption. Polyphosphates did not appear to be related to light and temperature stress, because no significant changes in polyphosphates were observed in cultured algae under light and temperature stress.

1. Introduction

Particulate organic matter (POM) in surface seawater, primarily a direct derivative of living and dead organisms, contains a wide range of chemical compounds. Relatively little is known about the chemical composition of POM pools, or the changes in composition of POM when species assemblage or environmental conditions are altered (Hedges et al., 2001). There is considerable evidence that phytoplankton species differ substantially in their biogeochemical characteristics (e.g. Healy and Hendzel, 1979; Shifrin and Chisholm, 1981; Arrigo et al., 1999; Xu et al., 2006). Additionally, phytoplankton will alter their cell physiology in response to environmental stress, including changes in light, temperature and nutrients. Factors affecting photosynthesis have long been known to alter C fixation and the allocation of C into macromolecules. As such, light intensity has been shown to alter proteins (Morris et al., 1974; Cuhel and Lean, 1987; Mock and Kroon, 2002; Khotimchenko and Yakovleva, 2005) and lipids (Mock and Gradinger, 2000; Mock and Kroon, 2002; Fábregas et al., 2004; Khotimchenko and Yakovleva, 2005). Temperature stress will also alter C allocation, particularly lipids (Al-Hasan et al., 1991; Kakinuma et al., 2001; Khotimchenko and Yakovleva, 2005; Ventura et al., 2008). Nutrient stress has been reported to affect C forms: N starvation may enhance lipid storage (Shifrin and Chisholm, 1981) and may decrease protein content (Kilham et al., 1997; Lynn et al., 2000; Heraud et al., 2005), while increased carbohydrate:protein ratios are thought to be indicative of P deficiency (Healy and Hendzel, 1979; Kilham et al., 1997; Dean et al., 2008). Phosphorus stress can also alter the response of algae to light and temperature stress (e.g. Gauthier and Turpin, 1997; Sterner et al., 1997).

Little is known about changes in plankton P forms with environmental stress. Many of these forms are important metabolically and structurally, such as DNA, sugar phosphates and phospholipids, and are organic compounds chemically linked to C. Thus, if C fixation is altered by environmental stress, then organic P forms could also be altered. The C:P ratio has been shown to remain close to the Redfield ratio under low-light conditions, but to increase under high light conditions (Sterner et al., 1997), but it is not known if this also produces changes in the distribution of organic P forms. Inorganic P forms, such as orthophosphate and polyphosphate, may also be altered by environmental stress. Indeed, in bacteria, polyphosphates and associate enzymes such as polyphosphate kinase are involved in a wide range of aspects of metabolism, and play an important role in responding to a variety of environmental stresses (Jahid et al., 2006; Manganelli, 2007; Brown and Kornberg, 2008). For algae, luxury accumulation of polyphosphate is well known (e.g. Droop, 1973; Stevenson and Stoermer, 1982; Sterner and Elser, 2002). With respect to stress, it appears that polyphosphate accumulation in *Dunaliella salina* can be altered by alkaline stress (Pick et al., 1990) and osmotic stress (Bental et al., 1991). However, there are few reports to indicate that changes in

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polyphosphate synthesis represent an environmental stress response in algae to the same extent as has been observed for bacteria.

The objective of this research was to examine changes in plankton allocation of C and P in response to light, temperature or P nutrition stress, using solid-state ^{13}C CPMAS and solution ^{31}P NMR spectroscopy to characterize C and P forms or compound classes in culture-grown samples. Different algal species were grouped together based on the applied stress for statistical analyses, to determine if broad trends in C and P allocations could be detected. This is an important first step in understanding the factors controlling the production of organic compounds, and ultimately the factors controlling the oceanic biological pump.

2. Materials and methods

2.1. Algal cultures

A number of algal species were grown in culture to elucidate the role of various environmental stressors on allocation and synthesis of various C and P forms. Unless otherwise noted, species were cultured in F/2 marine phytoplankton culture medium (Sigma) added to standard artificial ocean water (Goldman and McCarthy, 1978) at 25 μmol photons $m^{-2} s^{-1}$, and 20 °C. These species included: *Thalassiosira pseudonana* (CCMP 1335), *T. weissflogii* (CCMP 1051), *Symbiodinium* sp. (CCMP 832), *Emiliania huxleyi* (CCMP 2090), *Dunaliella tertiolecta* (CCMP 1320), *Synechococcus* sp. (CCMP 2370), *Amphora salina, Imantonia rotunda, Fragilariopsis curta, F. cylindrus, Nitzschia subcurvata* and *Phaeocystus antarctica*. These species include diatoms (*T. pseudonana*, *T. weissflogii*, *A. salina*, *F. curta*, *F. cylindrus*, and *N. subcurvata*), a prymnesophyte (*P. antarctica*), a coccolithophore (*E. huxleyi*), a chlorophyte (*D. tertiolecta*), a haptophyte (*I. rotunda*), a dinoflagellate alga (*Symbiodinium* sp.) and a cyanobacterium (*Synechococcus* sp.). The species grown under low-temperature (10 and 15 °C) stress and control temperature (20 °C) were *A. salina* (10 and 20 °C) and *E. huxleyi* (10, 15 and 20 °C), constituting two species for the control and three comparison samples (two species, one grown at two different low temperatures) for the treatment. The species grown under high temperature (26 °C) stress and control temperature (20 °C) were *T. weissflogi*, *D. tertiolecta* and *Synechococcus* sp., with three comparison samples each used for the control and for the high-temperature treatment. The species grown under high-P (7.2 μM mol P) and control P (3.6 μM mol P) conditions were *A. salina*, *E. huxleyi*, *I. rotunda*, *T. pseudonana*, and *Synechococcus* sp., with five comparison samples each for control and treatment. The species grown under low-P (0.5 μM mol P) and control P (3.6 μM mol P) conditions were *E. huxleyi*, *Symbiodinium* sp., *T. pseudonana*, and *Synechococcus* sp., with five comparison samples (one species was cultured twice) each for control and treatment. Control light conditions were 25 or 125 μmol photons $m^{-2} s^{-1}$, depending on the species, high light conditions were 250 or 125 μmol photons $m^{-2} s^{-1}$, depending on the species, and low light conditions were 5 or 25 μmol photons $m^{-2} s^{-1}$, depending on the species. The species grown under low light and controlled light were *F. curta*, *F. cylindrus*, *N. subcurvata*, *P. antarctica*, *T. pseudonana*, *E. huxleyi* and *Symbiodmium* sp., with seven samples for control and eight comparison samples (one species cultured twice) grown under low light. The four species grown under high light and control conditions were *F. curta*, *F. cylindrus*, *N. subcurvata*, and *P. Antarctica* (four samples each for high light and control).

2.2. Chemical analyses and NMR spectroscopy

Total C and total N were analyzed on a Carlo Erba NA1500 Series II elemental analyzer, without acidification. Total P was determined by digestion (Parkinson and Allen, 1975; O'Halloran and Cade-Menun, 2008), followed by colorimetric analysis (Murphy and Riley, 1962).

2.3. ^{13}C NMR spectroscopy

Solid-state cross-polarizing magic angle spinning (CP/MAS) spectra were acquired at a ^{13}C frequency of 100.53 on a Varian VXR/Unity spectrometer with a wide-bore 9.4T magnet and a 7 mm Jakobsen probe. Oven-dried samples (0.052–0.249 g) were packed into a 7-mm silicon nitride rotor with a Kel-F end cap, and were spun at 6000 ± 10 Hz. For small samples, a spacer of hexagonal boron nitride was inserted into the rotor prior to packing the samples, to properly align them in the center of the rotor. A standard cross-polarization sequence was used, including a pulse length of 4.0 μs, a 0.5-s recycle delay, and a spectral width of 100,000 Hz. The number of scans acquired ranged from 25,860 to 483,424, depending on the sample size and C concentration. Chemical shifts were externally referenced to the methyl resonance of hexamethylbenzene at 17.36 ppm (Hedges et al., 2001).

Carbon NMR spectra were processed using NUTS software (Acorn NMR, Livermore CA), with a backward linear prediction of 2 points and a 200-Hz Gaussian multiplication Fourier transformation of the acquired FID. The spectra were baseline corrected between −40 and 300 ppm, and the rotor background signal was subtracted after processing. The spectra were divided into seven spectral regions (Hedges et al., 2002; Nelson and Baldock, 2005): I, 0–45 ppm (alkyl C); II, 45–60 ppm (N-alkyl and methoxyl C); III, 60–95 ppm (O-alkyl C); IV, 95–110 (di-O-alkyl C); V, 110–145 ppm (unsaturated C); VI, 145–165 ppm (O-Aromatic C); and VII, 165–220 ppm (carbonyl C). Example spectra are shown in Fig. 1. The distribution of ^{13}C NMR signal intensity was quantified by determining the relative contributions of total signal intensity associated with each spectral region (%). Spectral intensities areas were corrected for spinning side bands (SSB) by adding two times the signal intensity associated with the prominent SSB at 230 ppm to the signal intensity of region VII, and subtracting half the signal intensity of the 230-ppm SSB from each of

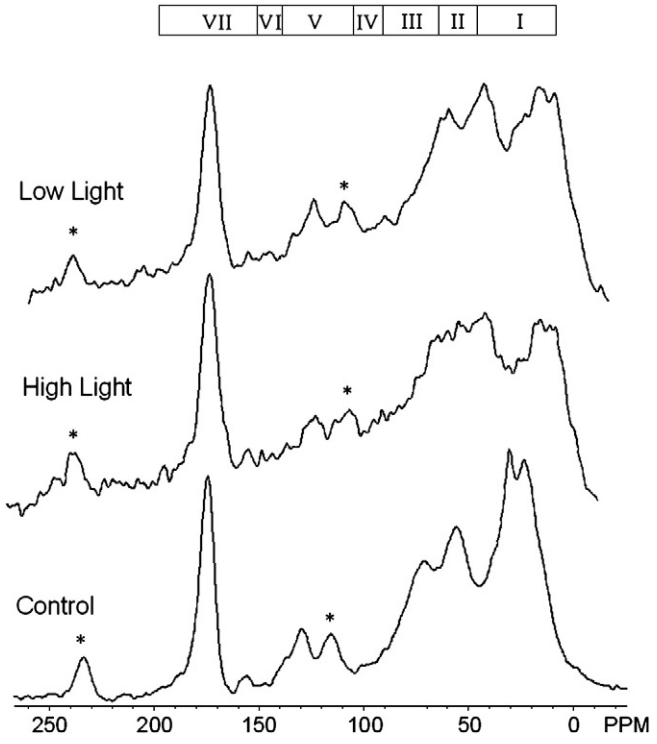

Fig. 1. Examples of ^{13}C CP/MAS NMR spectra for *Fragilariopsis cylindrus* grown under control (125 μmol photons $m^{-2} s^{-1}$), high (250 μmol photons $m^{-2} s^{-1}$) and low (5 μmol photons $m^{-2} s^{-1}$) light conditions. Roman numerals indicate C form classes I, 0–45 ppm (alkyl); II, 45–60 ppm (N-alkyl); III, 60–95 ppm (O-alkyl); IV, 95–110 (di-O-alkyl); V, 110–145 ppm (C C*–H (or –C)); VI, 145–165 ppm (C C*–O (or –N)); VII, 165–220 ppm (carbonyl). An * indicates a spinning side band.