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Production of glycine betaine and dimethylsulfoniopropionate in marine phytoplankton. II. N-limited chemostat cultures

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Abstract The nitrogenous organic osmolyte glycine betaine (GBT) and its sulfur analog dimethylsulfoniopropionate (DMSP) are quantitatively significant solutes in many marine algae. Although an inverse relationship has been suggested between these two compounds in marine phytoplankton that may be regulated by nitrogen availability, our results in Part I of this work (same issue) showed no such relationship in batch cultures of six species. In experiments conducted in August 1994, we reexamined this relationship in three axenic strains of phytoplankton [*Thalassiosira pseudonana* (Hustedt) Hasle et Heimdal, *Emiliania huxleyi* Hay et Mohler, and *Amphidinium carterae* Hulbert] in chemostat cultures at three N-limited growth rates. Levels of DMSP, GBT, and homarine, another nitrogenous osmolyte, were inversely related in *T. pseudonana*, with GBT and homarine preferentially produced at the highest growth rate when cells were N-replete. DMSP concentrations did not change in *E. huxleyi*, although GBT and homarine levels did increase at the highest growth rate. In *A. carterae*, neither DMSP nor GBT varied in any systematic way. In all three algae, additions of nitrogen to N-limited cultures resulted in short-term increases in GBT production. Levels of cellular DMSP remained fairly constant in *E. huxleyi* and *A. carterae* and were much greater than levels of comparable nitrogenous osmolytes like GBT and homarine. The dominance of DMSP makes a reciprocal relationship with the nitrogenous osmolytes unlikely in most species. Phytoplankton appear to be capable of directly assimilating extracellular GBT, although it is not known if they eliminate equivalent amounts of DMSP in the process.

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Introduction

Marine phytoplankton produce a variety of organic nitrogen- and sulfur-containing osmolytes that may form a significant percentage of the respective organic nitrogen and sulfur pools in phytoplankton and of pelagic ecosystems in general. These osmolytes include the quaternary ammonium compound glycine betaine (GBT) and the tertiary sulfonium compound dimethylsulfoniopropionate (DMSP). The production, distribution, and cycling of these compounds in the sea are poorly understood. While DMSP has been quantified in phytoplankton cultures (e.g. Keller et al. 1989) and field samples (e.g. Turner et al. 1988), only a limited amount of information is available about which phytoplankton species produce significant quantities of GBT. It is known that some algae produce both GBT and DMSP (Blunden and Gordon 1986; Dickson and Kirst 1987a, b) and that the relative proportions of GBT and DMSP are not necessarily constant, as was observed in cells of *Platymonas subcordiformis* grown at different salinities (Dickson and Kirst 1987a). In addition, several studies have noted that intracellular levels of DMSP in many marine phytoplankton species are substantially lower in N-replete batch cultures than in those that are N-limited (Turner et al. 1988; Gröne and Kirst 1992; Keller and Bellows 1996).

DMSP and GBT are energetically expensive molecules to synthesize, each ultimately requiring reductive assimilation of sulfate or nitrate, and several subsequent methylations. The synthesis and maintenance of these compounds in the cells are therefore likely to be under strict control. In the case of GBT, the allocation of a large part of cellular nitrogen reserves for osmotic purposes is particularly interesting because nitrogen is considered to be limiting in many marine ecosystems

(Ryther and Dunstan 1971; Sharp 1983). In this regard, Andreae (1986) proposed that a sulfur osmolyte such as DMSP may be favored over N-containing osmolytes in oceanic phytoplankton, due to the overwhelming abundance of sulfate in seawater (~28 m*M*) as compared to combined nitrogen (1 to 10 μ*M*). Replacement of a N-containing molecule with a functionally similar one containing sulfur would allow the scarce nitrogen to be used for other vital molecules such as amino acids, membrane lipids, and nucleotides. Andreae postulated that the relatively high production of DMS in oligotrophic waters could be due to the preferential use of DMSP by the N-limited phytoplankton community. Variable DMSP and GBT production by phytoplankton in response to N availability may influence the oceanic production of DMS.

In a previous study, we measured these compounds over the growth cycle in batch cultures of six strains of marine phytoplankton (Keller et al. 1999). All six species produced DMSP, while three produced GBT at lesser levels. When present, GBT formed a sizeable fraction of the cellular nitrogen only during exponential growth. There did not appear to be a reciprocal relationship between DMSP and GBT production, although GBT production does appear to be correlated with N availability. Since previous studies have observed an inverse relationship between N limitation and levels of DMSP, it is still not clear how the cycling of these compounds is related. The lack of knowledge about the possible alternation between DMSP and GBT in phytoplankton represents an important gap in our understanding of the biogeochemical cycling of N and S.

In the present study, we investigated the production of GBT and DMSP by selected species of marine phytoplankton in N-limited chemostat cultures. Previous studies examined production of these compounds using batch cultures, but the uncertainties associated with conditions in batch cultures (i.e. unbalanced growth, lack of information on nutrient limitation) necessitated the use of the controlled conditions found in chemostat culture (i.e. balanced growth, ability to control growth rate with single, known limiting nutrient). We were particularly interested in determining whether the levels of GBT in phytoplankton were a function of N availability and, for species that produce both GBT and DMSP, if alternative use of DMSP would be favored under N limitation.

Materials and methods

Three species of phytoplankton were used in these experiments conducted in August 1994: a dinoflagellate, *Amphidinium carterae* (CCMP 1314), a prymnesiophyte, *Emiliania huxleyi* (CCMP 378), and a diatom, *Thalassiosira pseudonana* (CCMP 1335). The strains were chosen because all three are known to produce both DMSP and GBT (Keller et al. 1999; Keller and Kiene unpublished) and because they were axenic. The strains were obtained from the Provasoli-Guillard National Center for the Culture of Marine Phytoplankton (CCMP, Bigelow Laboratory, Maine, USA) and tested with standard bacterial media for contamination. The inocula cultures were grown at modest levels of nitrate (50 μ*M*) and after inoculation, 2-liter chemostats were grown as batch cultures until the cell numbers reached 10^5 cells ml^{-1}; then the medium was pumped from a sterile reservoir. The medium was a modified artificial seawater (AK) (Keller et al. 1987) with nitrate levels at 100 μ*M*. The chemostats were maintained at 20 °C and on a 14 h light:10 h dark cycle (at 1.5×10^{16} quanta cm^{-2} s^{-1}). The cultures were stirred with a floating Teflon stir bar and were aerated through a 0.2 μm filter capsule. Dilution rates were calculated from the volume of effluent collected daily and were controlled by a multichannel Manostat peristaltic pump. Constant volume was maintained via an overflow tube as new medium was introduced. Sampling occurred in mid-morning at the same time each day; samples were collected aseptically through a sampling port.

Cell growth was monitored daily by taking subsamples to measure relative in vivo fluorescence with a Turner Designs 100 fluorometer (Brand et al. 1981) and to determine cell number. Once growth had stabilized, the chemostats were sampled three times before a new growth rate was established. Three flow rates were chosen to obtain three different growth rates for each species. Six chemostat cultures were established simultaneously, one at the low and one at the medium growth rate for each alga. The flow rates were subsequently adjusted in the medium growth rate chemostats to produce the high growth rate conditions. Growth rates were calculated using the methods and assumptions established by Droop (1974). In addition, after completion of sampling, the low flow rate (most N-limited) chemostat was spiked with *K*-levels (883 μ*M*) of nitrate and sampled over time (25 h) to examine short-term responses to N additions.

The ability of the phytoplankton strains to take up dissolved GBT from the medium was also tested. Subsamples of the high growth rate chemostats were taken and incubated in flasks (14 h light:10 h dark cycle; 20 °C; no aeration) with and without an addition of GBT (50 μ*M*) to the culture medium. Samples were taken over time, filtered onto GF/F filters, and analyzed for intracellular GBT concentrations [GBT(P)].

Chemostat samples were taken for the following analyses:

- intra- and extracellular concentrations of dimethylsulfoniopropionate (DMSP),
- dimethyl sulfide (DMS),
- intracellular concentrations of glycine betaine (GBT) and other nitrogenous osmolytes,
- particulate organic sulfur (POS),
- particulate organic carbon and nitrogen (POC and PON),
- total protein and amino acids (AA),
- chlorophyll (chl), cell counts, and volumes, and
- nutrients in medium and intracellular inorganic nitrogen.

All analyses were as described in Keller et al. (1999). Cultures were tested every few days for the presence of bacteria using standard test media [*f*/2p (Guillard 1975) and M (Sieburth and Keller 1991)].

Results

Each chemostat was sampled three times at each growth rate, with the exception of the *Amphidinium carterae* culture with the highest growth rate, which was only sampled twice due to the appearance of a fungal contaminant. All of the other cultures remained axenic throughout the experiments. Growth rates were equivalent for all three algae; cell concentrations were about an order of magnitude higher in the *Thalassiosira pseudonana* and *Emiliania huxleyi* than in the *A. carterae* cultures (Tables 1 to 3). Cellular volumes decreased with growth rate in all three algae, as did chlorophyll, protein, and amino acid levels (Tables 1 to 3). It was

Table 1 *Thalassiosira pseudonana*. Variations in cell density and chemical composition of cells with growth rate. Number of replicates in brackets after experiment number. Mean values, followed by standard deviation in parentheses (*chl a* chlorophyll *a*; *POC*, *PON*, *POS* particulate organic carbon, nitrogen, and sulfur, respectively; *AA* amino acids; *DMSP* dimethylsulfoniopropionate; *GBT* glycine betaine)

Parameter	Experiment		
	1 [3]	2 [3]	3 [3]
Growth rate	0.8	0.5	0.3
Cell conc. (no. ml^{-1})	1.6×10^6 (0.2)	1.2×10^6 (0.1)	1.2×10^6 (0.1)
Cell volume (μm^3)	138.7 (46.1)	74.8 (28.6)	89.3 (32.6)
Chl *a* (pg $cell^{-1}$)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)
Protein (pg $cell^{-1}$)	2.2 (0.1)	1.9 (0.3)	1.7 (0.3)
Amino acids (pg $cell^{-1}$)	1.6 (1.3)	1.5 (1.6)	0.9 (0.1)
POC (pg $cell^{-1}$)	8.7 (1.5)	7.3 (1.5)	9.0 (1.7)
PON (pg $cell^{-1}$)	1.3 (0.6)	1.0 (0.0)	1.0 (0.0)
POS (pg $cell^{-1}$)	0.1 (0.02)	0.1 (0.04)	0.2 (0.03)
C:N	7.1 (0.5)	11.8 (0.1)	13.9 (0.3)
C:S	86.7 (22.9)	61.1 (25.9)	56.3 (28.7)
C:chl	61.9 (37.9)	91.6 (10.0)	150.0 (16.9)
AA:protein	0.7 (0.6)	0.8 (0.8)	0.5 (0.1)
µmol DMSP cm^{-3} cell vol.	0.9 (0.4)	23.4 (3.6)	25.9 (0.6)
µmol GBT cm^{-3} cell vol.	2.0 (1.7)	0.9 (0.5)	0.2 (0.2)
µmol homarine cm^{-3} cell vol.	0.2 (0.2)	0.4 (0.1)	0.02 (0.02)
DMSP(S) as % of total S	4.4 (2.0)	47.0 (14.6)	46.6 (15.5)
Protein(S)[a] as % of total S	20.0 (4.0)	15.8 (4.3)	10.9 (3.8)

[a] Based on protein(S) estimate as 1% of total protein

Table 2 *Emiliania huxleyi*. Variations in cell density and chemical composition of cells with growth rate. Number of replicates in brackets after experiment number. Mean values, followed by standard deviation in parentheses. Abbreviations as for Table 1

Parameter	Experiment		
	1 [3]	2 [3]	3 [3]
Growth rate	0.8	0.4	0.3
Cell conc. (no. ml^{-1})	1.1×10^6 (0.1)	1.1×10^6 (0.1)	1.4×10^6 (0.1)
Cell volume (μm^3)	65.5 (0.0)	36.1 (19.2)	38.4 (16.7)
Chl *a* (pg $cell^{-1}$)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)
Protein (pg $cell^{-1}$)	2.6 (0.1)	2.0 (0.4)	1.7 (0.2)
Amino acids (pg $cell^{-1}$)	1.7 (0.2)	0.9 (0.1)	0.8 (0.4)
POC (pg $cell^{-1}$)	8.7 (0.6)	8.3 (1.5)	10.7 (2.1)
PON (pg $cell^{-1}$)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)
POS (pg $cell^{-1}$)	0.1 (0.02)	0.1 (0.02)	0.2 (0.02)
C:N	6.9 (0.9)	10.5 (0.4)	14.7 (0.7)
C:S	71.1 (28.1)	64.1 (6.8)	71.1 (23.5)
C:chl	66.7 (6.4)	104.1 (24.6)	177.8 (64.7)
AA: protein	0.7 (0.1)	0.5 (0.1)	0.5 (0.3)
µmol DMSP cm^{-3} cell vol.	59.0 (10.2)	110.8 (5.1)	90.0 (12.0)
µmol GBT cm^{-3} cell vol.	0.5 (0.4)	0.0 (0.1)	0.0 (0.1)
µmol homarine cm^{-3} cell vol.	0.3 (0.3)	0.2 (0.1)	0.1 (0.1)
%DMSP(S):POS[a]	103.9 (58.5)	98.8 (6.5)	74.4 (11.7)
Protein(S)[b] as % of total S	21.6 (7.2)	15.5 (3.0)	11.1 (1.8)

[a] Ca. 20% of DMSP is volatilized and lost during POS analysis (Matrai and Keller 1994)
[b] Based on protein(S) estimate as 1% of total protein

apparent that the cultures were more N-deplete at lower growth rates, as C:N and C:chl ratios increased with decreasing growth rate (Tables 1 to 3). Amino acid:protein ratios also decreased as the cells became more N-deplete (Tables 1 to 3).

All three species produced DMSP (Tables 1 to 3), with intracellular levels of DMSP ranging from 1 to 125 µmol DMSP based on live cell volumes (Tables 1 to 3). The greatest cellular concentrations occurred in *Emiliania huxleyi* (CCMP 378) and *Amphidinium carterae* (CCMP 1314), and levels remained fairly stable among the different growth rates (Tables 2, 3). DMSP levels were low and declined as growth rate increased in the diatom *Thalassiosira pseudonana* (CCMP 1335) (Table 1). DMS and DMSP(D) production were insignificant at all growth rates in all strains (data not shown).

The nitrogenous osmolytes GBT and homarine were produced in low concentrations in all three algae (no homarine in the dinoflagellate) (Tables 1 to 3). In *Thalassiosira pseudonana* (CCMP 1335) and *Emiliania huxleyi* (CCMP 378), levels of GBT and homarine were inversely related to growth rate (Tables 1, 2). No pattern was evident in the production of GBT in *Amphidinium carterae* (CCMP 1314) (Table 3). DMSP concentrations were up to an order of magnitude higher than the combined nitrogenous osmolytes in the diatom and prymnesiophyte and three orders of magnitude higher in

Table 3 *Amphidinium carterae*. Variations in cell density and chemical composition of cells with growth rate. Number of replicates in brackets after experiment number. Mean values, followed by standard deviation in parentheses. Abbreviations as for Table 1

Parameter	Experiment		
	1 [2]	2 [3]	3 [3]
Growth rate	0.7	0.4	0.2
Cell conc. (no. ml^{-1})	4.5×10^4 (1.5)	8.1×10^4 (0.9)	7.4×10^4 (1.2)
Cell volume (μm^3)	795.2 (429.2)	505.6 (158.3)	549.9 (117.7)
Chl *a* (pg $cell^{-1}$)	1.5 (0.1)	0.7 (0.1)	0.7 (0.0)
Protein (pg $cell^{-1}$)	42.4 (5.9)	26.4 (3.8)	27.5 (4.1)
Amino acids (pg $cell^{-1}$)	31.6 (5.4)	5.9 (2.1)	8.5 (5.6)
POC (pg $cell^{-1}$)	121.0 (28.3)	82.3 (9.1)	128.7 (11.2)
PON (pg $cell^{-1}$)	18.0 (4.0)	9.7 (1.1)	11.0 (1.6)
POS (pg $cell^{-1}$)	2.4 (0.8)	1.5 (0.3)	2.3 (0.8)
C:N	6.8 (0.2)	8.6 (0.1)	11.9 (0.3)
C:S	50.2 (8.1)	53.8 (4.6)	56.0 (24.7)
C:chl	78.6 (0.8)	116.0 (22.9)	192.0 (45.6)
AA: protein	0.7 (0.2)	0.2 (0.1)	0.3 (0.2)
μmol DMSP cm^{-3} cell vol.	116.5 (8.8)	125.3 (7.7)	117.6 (11.9)
μmol GBT cm^{-3} cell vol.	0.2 (0.6)	0.02 (0.0)	0.1 (0.3)
μmol homarine cm^{-3} cell vol.	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
DMSP(S) as % of total S[a]	123.6 (87.7)	133.0 (23.3)	90.4 (58.4)
Protein(S)[b] as % of total S	17.6 (3.1)	17.3 (5.5)	12.0 (3.8)

[a] Ca. 20% of DMSP is volatilized and lost during POS analysis (Matrai and Keller 1994)
[b] Based on protein(S) estimate as 1% of total protein

the dinoflagellate. No trigonelline was observed in any of these cultures.

An ANOVA was performed on the production of osmolytes versus growth rate for each alga (Table 4). In *Thalassiosira pseudonana,* differences in DMSP levels were highly significant between all growth rates, as were GBT levels between the high versus mid and high versus low growth rates, but not between low and mid rates. No significant differences were present in DMSP levels between growth rates in *Emiliania huxleyi,* but significant differences were present in GBT levels between high versus mid and high versus low growth rates, but not between low and mid rates. Significant differences were also apparent in homarine levels between high versus low growth rates, but not between high versus mid or low versus mid rates in *E. huxleyi*. In *Amphidinium carterae,* DMSP levels were significantly different between high versus mid and high versus low growth rates, but not between low and mid rates, but there were no significant differences in GBT between any growth rates.

DMSP comprised a significant percentage of the cellular organic sulfur in all three species, ranging from 46 to 100% of the measured POS, except for *T. pseudonana* at the highest growth rate where DMSP comprised only 4% of POS (Tables 1 to 3). GBT and homarine did not form a sizeable fraction of the cellular nitrogen in any of

Table 4 Analysis of variance of N-limited growth rates versus production of compounds in three phytoplankton strains. Units are fM(compound) $cell^{-1}$ (*df* degrees of freedom; *SS* sum of squares; *MS* mean square error)

Strain	Source of variation	*df*	SS	MS	*F*	P-value
Thalassiosira pseudonana (DMSP)	Between groups	2	7.72	3.86	150.17	0.00001
	Within groups	6	0.15	0.03		
	Total	8	7.87			
T. pseudonana (GBT)	Between groups	2	0.229	0.115	6.371	0.0099
	Within groups	15	0.270	0.018		
	Total	17	0.499			
Emiliania huxleyi (DMSP)	Between groups	2	0.46	0.23	1.02	0.416
	Within groups	6	1.36	0.23		
	Total	8	1.82			
E. huxleyi (GBT)	Between groups	2	0.002	0.001	6.653	0.0085
	Within groups	15	0.003	0.000		
	Total	17	0.005			
E. huxleyi (homarine)	Between groups	2	0.002	0.001	5.350	0.0176
	Within groups	15	0.002	0.000		
	Total	17	0.004			
Amphidinium carterae (DMSP)	Between groups	2	1235.08	617.54	18.70	0.0048
	Within groups	5	165.11	33.02		
	Total	7	1400.20			
A. carterae (GBT)	Between groups	2	0.096	0.048	0.822	0.4613
	Within groups	13	0.757	0.058		
	Total	15	0.853			

Table 5 Relative contributions of inorganic N pools, amino acids, proteins, GBT and other nitrogenous osmolytes to total cellular organic nitrogen pools in each phytoplankton strain at three growth rates. Mean values, followed by standard deviation in parentheses

Strain (CCMP No.)	Growth rate	% NO_3	% NH_4	% GBT	% Homarine	% Amino acids	% Protein	% Cum.
T. pseudonana (1335)	0.78	0.45 (0.52)	0.42 (0.37)	0.36 (0.25)	0.03 (0.03)	12.49 (9.95)	25.47 (7.72)	39.22
	0.45	0.29 (0.13)	2.78 (1.28)	0.09 (0.05)	0.03 (0.01)	8.38 (2.70)	25.61 (5.05)	37.19
	0.28	0.20 (0.09)	2.10 (0.09)	0.03 (0.03)	0.02 (0.00)	10.11 (4.55)	22.69 (12.77)	35.10
E. huxleyi (378)	0.82	0.10 (0.00)	3.38 (1.08)	0.05 (0.02)	0.05 (0.02)	12.81 (3.78)	36.98 (2.35)	53.37
	0.41	0.13 (0.03)	2.26 (0.74)	0.00 (0.00)	0.01 (0.01)	7.92 (1.30)	26.55 (2.13)	36.86
	0.30	0.10 (0.02)	1.44 (0.48)	0.00 (0.00)	0.01 (0.00)	6.98 (5.65)	24.27 (1.92)	32.77
A. carterae (1314)	0.74	0.28 (0.14)	3.54 (1.31)	0.02 (0.03)	0.00 (0.00)	14.36 (3.11)	29.61 (14.03)	47.81
	0.42	0.11 (0.02)	2.30 (0.51)	0.01 (0.00)	0.00 (0.00)	7.26 (2.16)	43.91 (4.12)	53.59
	0.20	0.14 (0.07)	2.26 (1.31)	0.02 (0.03)	0.00 (0.00)	4.22 (1.92)	37.71 (9.78)	44.36

these experiments (Table 5). The highest contribution of GBT to cellular nitrogen ($<0.4\%$) occurred at the highest growth rate (most N-replete) of the *T. pseudonana* (CCMP 1335) culture. A significant percentage (ca. 50%) of the organic nitrogen could not be accounted for in these algae even when cellular pools of protein, amino acids, inorganic nitrogen, and nitrogenous osmolytes were combined (Table 5).

In separate experiments, the most N-deplete (lowest growth rate) chemostats were spiked with a NO_3^- addition and sampled over time. The C:N ratio decreased in all three species, demonstrating uptake and incorporation of nitrogen (Fig. 1A to C). In response, the intracellular levels of DMSP dropped in *Thalassiosira pseudonana* and *Amphidinium carterae*. Levels of GBT increased in all three species over time, and homarine increased in *T. pseudonana* and *Emiliania huxleyi*.

In separate subcultures of each species, GBT (50 μM) was added to the culture medium and GBT concentration within the cells was monitored. In each case, intracellular levels of GBT increased while controls (no GBT additions) remained constant (Fig. 2A to C).

Discussion

DMSP, GBT, and other nitrogenous osmolytes are highly dynamic compounds within marine phytoplankton, with their production being species- and perhaps strain-specific and dependent on physiological state. The levels of the nitrogenous compounds appear to be particularly variable and a function of N availability. The relationship between DMSP and nitrogen does not appear to be straightforward.

Measurements of physiological indicators of N limitation: increasing C:N ratios, increasing C:chl ratios, and decreasing AA:protein ratios all point to cells experiencing increasing N limitation with decreasing growth rate. Although, the experiments performed in this study were in continuous cultures (i.e. balanced growth and nitrogen limitation), and were thus fundamentally different from those in a previous study where batch cultures were employed (i.e. unbalanced growth and nutrient starvation) (Keller et al. 1999), the cellular ratios we observed in both studies were very comparable.

Levels of DMSP were also very comparable to previous observations (Keller 1991; Matrai and Keller 1994; Keller and Bellows 1996). Intracellular levels of DMSP in *Thalassiosira pseudonana* decreased sharply at the fastest growth rate, but DMSP concentrations did not decrease with increasing N availability in the other two species. This is contrary to earlier reports of enhanced levels of DMSP in phytoplankton batch cultures that were N-deplete (Turner et al. 1988; Gröne and Kirst 1992; Keller and Bellows 1996). GBT and the nitrogenous osmolyte homarine were present in all three algal strains examined in this study, with the exception of homarine in *Amphidinium carterae*. In all cases, the presence of these nitrogenous compounds appeared to be related to N availability (i.e. highest levels at highest growth rate).

The proposed reciprocal relationship between DMSP and GBT, suggested by Andreae (1986) in marine phytoplankton, was evident in only one of the three algae used in this study. In *Thalassiosira pseudonana*, DMSP production did appear to be favored over that of N-containing osmolytes when nitrogen was most limiting, and likewise, GBT and homarine were preferentially produced over DMSP at the highest growth rate when cells had the most nitrogen. The other two phytoplankton species examined continued to produce DMSP regardless of N availability, although GBT and homarine in these cultures were sensitive to N availability and formed maximum levels when cells were most N sufficient. Levels of DMSP within most phytoplankton species appear to remain fairly constant and are typically much higher than levels of comparable nitrogenous osmolytes such as GBT and homarine. It is perhaps noteworthy that it was only in the diatom, where concentrations of DMSP and the nitrogenous osmolytes were within the same order of magnitude, that a reciprocal relationship existed. In algae, where DMSP is the primary osmolyte, variations are not as evident and pools not as dynamic. Marine organisms utilize a suite of different compounds to maintain osmotic balance. These include inorganic ions, especially K^+, polyols such as glycerol or mannitol, sugars, amino acids, and more complex molecules such as DMSP and GBT (Kirst 1996 and references within). While DMSP was present in osmotically significant amounts in the prymnesiophyte and dinoflagellate, none of the nitrogenous osmolytes were abundant enough to affect

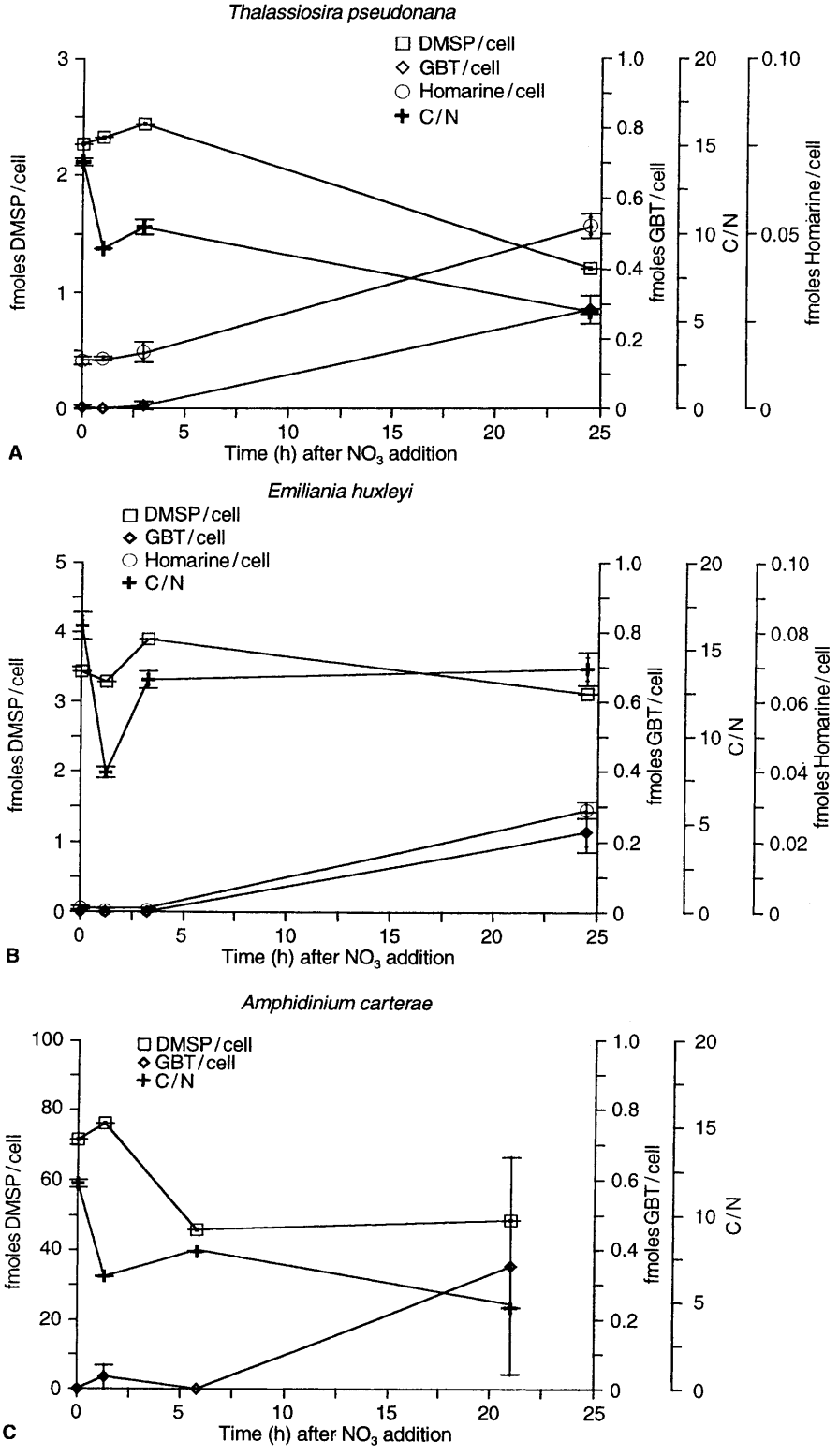

Fig. 1 *Thalassiosira pseudonana* (**A**), *Emiliania huxleyi* (**B**), *Amphidinium carterae* (**C**). Temporal changes in intracellular concentrations of DMSP, GBT, and homarine, as well as C:N ratios, in response to a N addition to a N-limited culture. Means ± 1 SD are shown for each datum

osmotic pressure significantly. Using the equations in Dickson and Kirst (1987a), we estimate that DMSP contributed up to 25% of the intracellular osmotic pressure in these cells. GBT accounted for < 1%. Thus, a reciprocal relationship between the two compounds is unlikely when one is so clearly dominant.

DMSP, as a percentage of the total organic sulfur, ranged from 46 to > 100% within all the phytoplankton strains, except for *Thalassiosira pseudonana* at the highest growth rate where DMSP(S) comprised only 4% of the POS (Tables 1 to 3). This range is similar to the observations of Matrai and Keller (1994) and

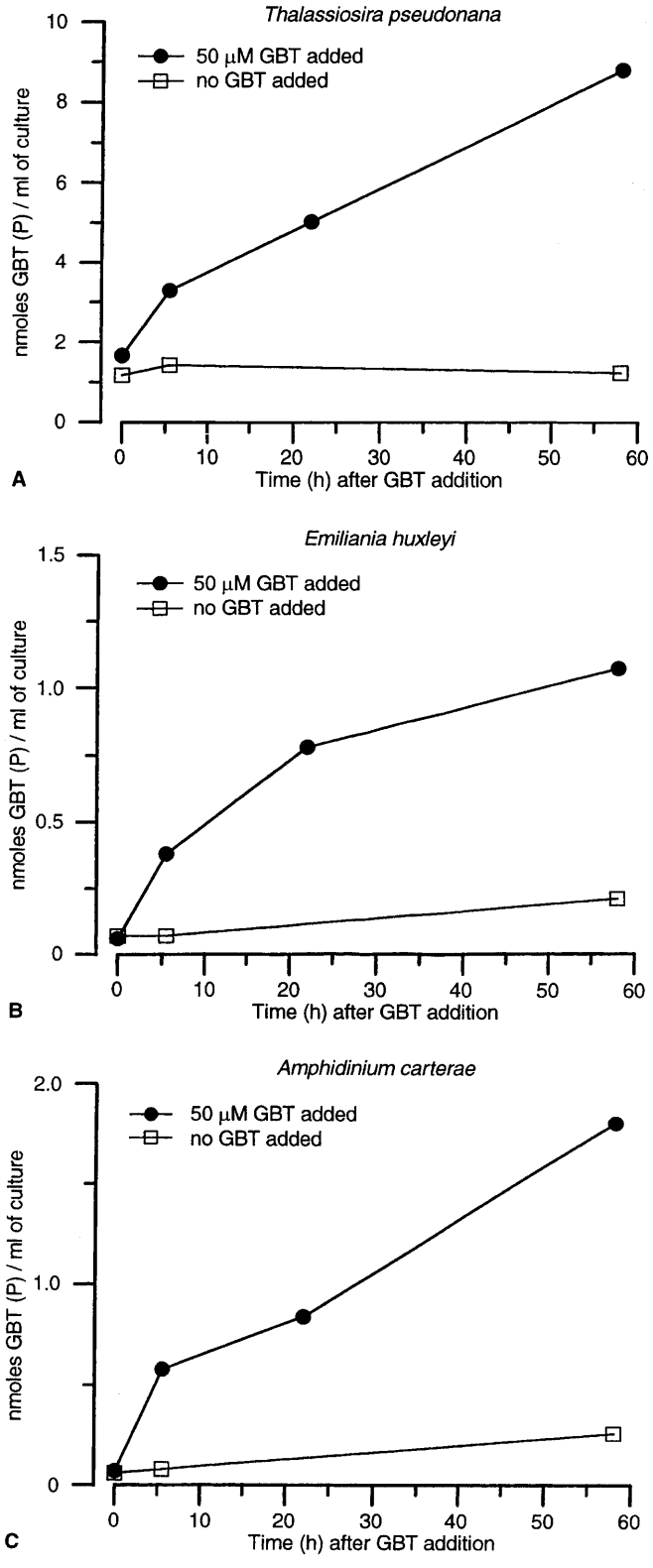

Fig. 2 *Thalassiosira pseudonana* (**A**), *Emiliania huxleyi* (**B**), *Amphidinium carterae* (**C**). Temporal changes in intracellular concentrations of GBT in cultures supplemented with 50 μ*M* GBT and with no GBT addition

Keller et al. (1999). We also estimated the percentage of sulfur that was contained in the protein fraction in these algae (Tables 1 to 3). The values ranged from 11 to 22% of the POS, assuming that ca. 1% of the measured protein was comprised of sulfur. This estimate was based on a comparison of C:S and C:N ratios in protein fractions from marine organisms (Cuhel et al. 1984) and the use of the %N of the protein standards. The contribution of protein (S) to the total organic sulfur in these algae is much lower than previous estimates (> 50%) (Andreae et al. 1990 and references therein). This discrepancy suggests that the protein fraction was underestimated.

We found that nitrogenous osmolytes contributed less than 1% of the cellular organic nitrogen, even when nitrogen was sufficient for high growth rates. Their presence appears to be more ephemeral than the amino acid pool, and is highly dependent on N availability. When individual N pools were summed in this study, it was clear that a large part of the cellular nitrogen in each alga was missing and not identifiable (Table 5). This phenomenon has been observed previously in marine phytoplankton (Conover 1975; Dortch et al. 1984; Doucette and Harrison 1991; Keller et al. 1999). In the present study, the contribution of the protein pool to total cellular nitrogen did not increase as the growth rate declined (most N-limited), although the amino acid pool did diminish somewhat with decreasing growth rate (Table 5). The contributions of protein to cellular nitrogen in this study were quite low in comparison with previous studies (e.g. Dortch et al. 1984; Keller et al. 1999), again suggesting that protein was underestimated.

The chemostat cultures used here were grown on a light:dark cycle because we have previously determined that levels of DMSP were up to 25% lower in *Amphidinium carterae* cells grown in continuous light (Keller unpublished). We recognize that, by introducing light:dark variability to the culture conditions, we have violated the criteria for chemostat culture. To minimize this flaw, the chemostats were sampled at the same time each day during the light period (mid-morning). We recognize that there is a known diel periodicity to N uptake in phytoplankton cultures. Eppley et al. (1971) and Eppley and Renger (1974) observed that uptake occurred primarily during the day, although only small diurnal changes were noted in intracellular nitrogen pools. In the present study, samples were only taken during the morning light period and, thus, there is no information on the presence of a diel pattern in any of the measured compounds' distributions. The observations in this study can only be said to be representative of mid-morning conditions and may or may not be generally representative.

Additions of NO_3^- to N-limited cultures resulted in increased GBT production in all three algae, and DMSP levels decreased in two species. DMSP did not decrease in *Emiliania huxleyi*. At the end of the 25 h incubation, levels of DMSP and GBT/homarine were roughly equivalent in *Thalassiosira pseudonana*, while in *E. huxleyi* and *Amphidinium carterae*, DMSP remained

one and two orders of magnitude, respectively, greater than the nitrogenous osmolytes.

In the experiment where we amended our cultures with GBT additions, GBT was apparently taken up directly by the cells. Uptake was much greater in the *Thalassiosira pseudonana* culture, again suggesting much greater potential cycling between GBT and DMSP in this alga. Unfortunately, we did not measure DMSP in these cultures at the same time, so it is unknown if DMSP was released in equivalent amounts. Kadota and Ishida (1968) observed that DMSP content in the dinoflagellate *Gyrodinium cohnii* was inversely related to GBT concentration, with cellular DMSP concentration decreasing by half when micromolar levels of GBT were added to the medium. Similarly, Kiene and Service (1993) demonstrated that the addition of 5 μM GBT to estuarine water samples resulted in a decrease in DMSP(P) production, with coincident increases in DMS and DMSP(D) in the water. Recent evidence (Hoffman 1996) suggested that some phytoplankton species (*Platymonas subcordiformis, Isochrysis galbana*) are capable of taking up even nanomolar levels of GBT.

Although there is no single, straightforward relationship between DMSP, GBT, and N availability, it is apparent that N- and S-containing osmolyte pools are dynamic, with phytoplankton cells actively releasing and taking up these compounds under certain conditions. DMSP is abundant and persistent in most marine phytoplankton, contributing significantly to organic sulfur pools and intracellular osmolarity. Nitrogenous osmolytes appear to be more ephemeral and to make a small, variable contribution to the nitrogen budget in cells. It has been shown previously that significant DMSP production is confined to a few classes of marine phytoplankton (Keller et al. 1989). The present study shows that N availability is also a contributing factor, especially in GBT production, in some algae. Class- or species-specific physiological characteristics interact with environmental factors, such as nutrient availability, in the oceanic sulfur cycle. As communities respond to changes in nitrogen, either physiologically or through species replacement, DMSP and perhaps DMS release will vary accordingly. It is still not possible to predict the patterns or magnitude of those responses based on any easily measured parameters, such as distributions of chlorophyll or inorganic nitrogen.

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