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34. We thank R. Britton, B. Marinovic, M. Moline, M. Putt, and M. Voytek for assistance in the field and D. Garrison, J. McClintock, R. Newell, and R. Olsen for comments on the manuscript. This work was supported by the National Science Foundation, Division of Polar Programs, grants DPP-8314607 (R.B.R.) and DPP-8317082 (J.S.P.) and by the University of Maryland, Center for Environmental and Estuarine Studies, contribution 1710.

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Oceanic Dimethylsulfide: Production During Zooplankton Grazing on Phytoplankton

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About half the biogenic sulfur flux to the earth's atmosphere each year arises from the oceans. Dimethylsulfide (DMS), which constitutes about 90% of this marine sulfur flux, is presumed to originate from the decomposition of dimethylsulfoniopropionate produced by marine organisms, particularly phytoplankton. The rate of DMS release by phytoplankton is greatly increased when the phytoplankton are subjected to grazing by zooplankton. DMS production associated with such grazing may be the major mechanism of DMS production in many marine settings.

DIMETHYLSULFIDE (DMS) IS UBIQUITOUS in the surface waters of the ocean and appears always to be present in concentrations far in excess of the concentrations expected at atmospheric equilibrium (*1*). This large concentration gradient between the oceans and the atmosphere drives almost half the biogenic sulfur flux to the earth's atmosphere (*2*). DMS constitutes about 90% of the flux of biogenic sulfur from the ocean to the atmosphere. As a result, considerable attention has been focused on the distribution and dynamics of DMS in ocean water in an effort to understand mechanisms controlling its flux to the atmosphere (*3, 4*).

The DMS in seawater appears to originate from algae, most likely produced by the decomposition of dimethylsulfoniopropionate (DMSP), a tertiary sulfonium compound analogous to the quaternary ammonium compounds (for example, glycinebetaine and proline) that are widespread in marine organisms (*5*). As with these ammonium compounds, DMSP may be involved in regulating cellular osmotic pressure in algae (*6, 7*). Decomposition of DMSP appears to occur mainly by an enzymatically catalyzed elimination reaction, yielding DMS and acrylic acid (*8*) (Fig. 1). First discovered in the marine alga *Polysiphonia fastigiata* by Challenger (*8*), DMSP has subsequently been documented in a wide range of marine algae (*9*). Many marine algae produce DMS in the normal course of metabolism (*7, 8*). Exact mechanisms and factors controlling DMSP decomposition in the ocean remain unknown.

Sieburth (*10*) reported that decomposition of DMSP originating in the alga *Phaeocystis* resulted in the accumulation of acrylic acid in the guts of penguins. We reasoned that, somewhere in the food chain, the decomposition of DMSP must also have resulted in loss of DMS to the water column. Furthermore, this process might occur throughout the marine food web. There is widespread evidence that zooplankton and other filter-feeding invertebrates ingest DMSP-containing plants with no adverse effects (*11*).

We investigated the production of DMS during grazing by the marine copepods *Labidocera aestiva* and *Centropages hamatus* on the dinoflagellate *Gymnodinium nelsoni*. Feeding experiments were conducted in silylated 1-liter glass bottles with silicone rubber stoppers containing 750 ml of filtered (5 μm) seawater. Four treatments were examined: with no organisms, with phytoplankton alone, with phytoplankton and zooplankton, and with zooplankton alone. We monitored DMS in the headspace of the bottles (*12*) to minimize disturbance to the plankton suspensions. Phytoplankton densities were set to 500 cells per milliliter, zooplankton to 30 to 40 animals per liter. These densities are higher than oceanic densities but occur in certain coastal situations. We selected these densities to ensure that measurements could be made over a 24-hour period. At the end of the experiment, the densities of phytoplankton and zooplankton were determined by direct counting.

The results of these experiments demonstrate that ingestion of phytoplankton by zooplankton releases DMS into the water column (Fig. 2). Using the weighted mean of linear least squares fits to the individual runs, we found that the rate of DMS production in bottles with zooplankton and phytoplankton averages 24 times that in bottles with phytoplankton alone. Bottles containing zooplankton alone and seawater without organisms showed no significant DMS production.

The weighted least squares slope for DMS concentration versus time in the phytoplankton bottles indicates a DMS production rate for the alga of 23×10^{-15} ($\pm 16 \times 10^{-15}$, 95% confidence) mol per cell per day. We know of only one other published estimate of DMS production by phytoplankton: *Hymenomonas carterae*, 1.3×10^{-15} mol per cell per day (at 35 parts per thousand) (*7*). The volume of cells of *G. nelsoni* ($\sim 2.4 \times 10^{-11}$ liter) is about 30 times that of *H. carterae* ($\sim 8 \times 10^{-13}$ liter), which may in part account for the difference in cell-specific rates of DMS production.

We estimated intracellular concentrations of DMSP by filtering known quantities of phytoplankton onto glass-fiber filters and treating the filters with base (*13*). The concentration of DMSP in *G. nelsoni* was about 280 mmol per liter of cell volume. In *H.*

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carterae, DMSP was about 120 mmol per liter of cell volume (*7*).

This experiment does not demonstrate the mechanism of DMS release. It may be that cells are damaged during capture and ingestion by zooplankton, or that DMSP decomposes during digestion in the intestinal tract of the zooplankton or by microbial activity in fecal material (*14*). We have determined that the gut contents of fed zooplankton are rich in DMSP. At the end of one experiment, the average zooplankter had a concentration of DMSP in its gut equivalent to 267 cells of *G. nelsoni*. When fed zooplankton were placed in alga-free water, DMS accumulated in the water, presumably the result of the continued digestion of DMSP or microbial degradation, or both. The concentration of DMSP in starved zooplankton was less than 4% of the amount in animals with full guts, so it does not appear that *L. aestiva* accumulates DMSP in body tissue.

On average, grazing by *L. aestiva* lowered phytoplankton densities in the bottles by 164 cells per milliliter. We can account for

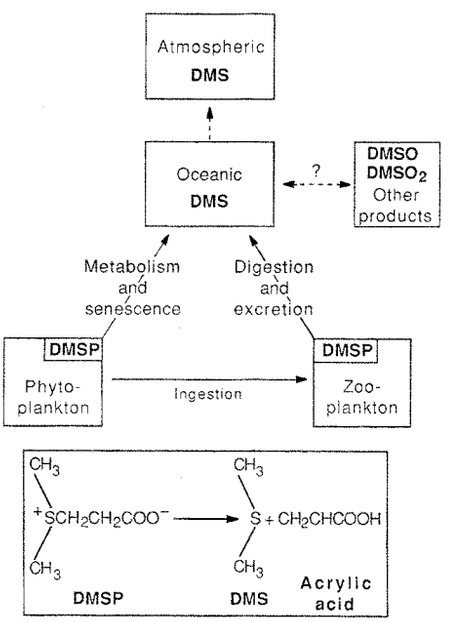

Fig. 1. Schematic of the effect of zooplankton grazing on DMS production in ocean water. DMSP in phytoplankton cells is slowly metabolized and DMS is released. As zooplankton ingest phytoplankton, DMS is released into the water column. The mechanism of DMS release is uncertain. Some DMS may be released from algae during capture and handling by zooplankton. DMS is also excreted or diffuses from zooplankton after ingestion. Some DMS may be oxidized in the zooplankton. DMS in ocean water may enter the atmosphere or may be oxidized in the water column to dimethylsulfoxide (DMSO), dimethylsulfone ($DMSO_2$), and other oxidation products. The DMS that escapes to the atmosphere from oceans represents about half the biogenic sulfur input to the atmosphere, or about one-fourth of the total flux of sulfur gas to the atmosphere.

37% of the DMSP in these cells as DMS free in solution. In the case of *C. hamatus*, 35% was recovered as DMS. We speculate that the remainder may have been oxidized to dimethyl sulfoxide or further decomposed by the zooplankton.

In an effort to estimate the potential significance of this mechanism to the marine DMS cycle, we made the following assumptions. On the basis of data for the two species of phytoplankton that have been studied, we assumed that the turnover rate for DMSP (releasing DMS) in DMSP-containing cells is 1% per day (*15*). We also assumed that, when such cells are ingested by zooplankton (regardless of species), one-third of each cell's DMSP would be released to the water as DMS. Under these circumstances, whenever more than 3% of DMSP-bearing phytoplankton in a parcel of water is ingested by zooplankton per day, the amount of DMS produced by ingestion should exceed that produced by phytoplankton alone (*15*).

The extent of zooplankton grazing in oceans has been a long-standing focus of research and debate, but it is almost certain that considerably more than 3% of phytoplankton cells are consumed on most days. In oceanic systems, zooplankton grazing usually matches phytoplankton production, keeping phytoplankton biomass in approximate steady state (*16*). At steady state, a phytoplankton production rate of 0.2 per day (*16*) would require that about 20% of phytoplankton cells be ingested each day. Under these circumstances, production of DMS during grazing should be six times the rate of DMS production by the phytoplankton alone. In coastal environments, zooplankton grazing may be less efficient at consuming primary production (*16*), and, if so, zooplankton would play a less important role in DMS production there than in areas of open ocean.

The mechanism for DMS production we have described should help explain some of the weakness in the relation between DMS concentrations and phytoplankton biomass. In general, DMS concentrations are higher where phytoplankton biomass is higher (*4*). Factoring in grazing by zooplankton should improve our understanding of the vertical profiles and diurnal dynamics in DMS in the water column.

Evaluation of this mechanism of DMS release into ocean water requires a better understanding of the physiology of phytoplankton and the factors controlling release of DMS by phytoplankton. The mechanisms of DMS release during ingestion by zooplankton also require further study. DMS may be released during capture and handling of phytoplankton cells by zooplankton, it may be excreted from or it may diffuse out of zooplankton, and it may be produced in fecal pellets. The relative importance of these mechanisms may influence the depth distribution of DMS production resulting from zooplankton grazing.

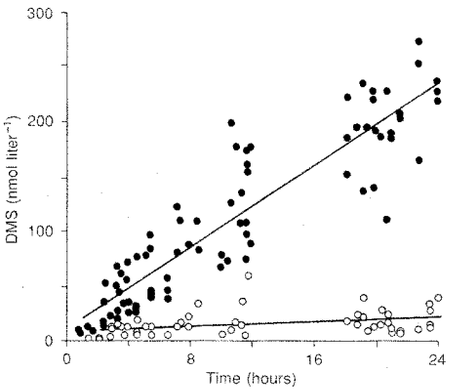

Fig. 2. Effect of zooplankton grazing on DMS release by phytoplankton: (●) zooplankton (*Labidocera aestiva*) + phytoplankton (*Gymnodinium nelsoni*); (○) phytoplankton alone. These data represent two separate runs with a total of nine bottles containing zooplankton and phytoplankton, and eight bottles containing phytoplankton alone. The weighted mean slopes are, respectively, 8.5 ± 0.6 and 0.35 ± 0.21 (95% confidence) nmol liter^{-1} hour^{-1}, yielding a ratio of slopes equal to 24.2 ± 14.4. Seawater and starved zooplankton produced no measurable DMS. Increased DMS production was also obtained with *L. aestiva* grazing on *Prorocentrum micans*, and the smaller copepod *Centropages hamatus* grazing on *G. nelsoni* and *P. micans*.

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12. The bottles were placed on a rotator (2 rev min^{-1}) in low light (3 to 20 μeinstein m^{-2} sec^{-1}; 12/12 light/dark) and 19°C. Samples were analyzed by cryofocusing on silylated glass beads in a liquid nitrogen bath, followed by chromatographic separation on a Chromosil 330 (Supelco) column with a flame photometric detector (Varian 3700) [see J. W. H. Dacey, S. G. Wakeham, B. L. Howes, *Geophys. Res. Lett.* **11**, 991 (1984)]. We prepared DMS standards by diluting a stock solution in filtered, gas-stripped seawater in identical 1-liter glass bottles. Headspace samples were withdrawn and analyzed in the same fashion.
13. The filters were placed in silylated serum tubes and stoppered with Teflon-faced stoppers; 2*N* KOH was added to the tubes, and the DMS headspace concentration was measured after 24 hours. DMSP is the only naturally occurring compound known to decompose to DMS in cold base (*8*).
14. Our unpublished data suggest that DMSP can be broken down to DMS by enzymes released during cell lysis. C. Wagner and E. R. Stadtman [*Arch. Biochem. Biophys.* **98**, 331 (1962)] and C. Wagner *et al.* [*J. Biol. Chem.* **241**, 1923 (1966)] described microbial degradation of DMSP to DMS by species of *Clostridium* and *Pseudomonas*.
15. In our experiment, daily DMS production in *G. nelsoni* equaled 0.3% of intracellular DMSP per day. We estimate from the data of Vairavamurthy *et al.* (*7*) that DMS production in *H. carterae* equaled about 1.4% of intracellular DMSP. DMS release is certainly dependent on physiological state, although Vairavamurthy *et al.* (*7*) suggest it is not a function of growth rate. All calculations are for DMSP-containing phytoplankton species only. Those phytoplankton without DMSP are not believed to produce DMS and are also unlikely to release DMS on ingestion.
16. For example, N. A. Welschmeyer and C. J. Lorenzen [*Limnol. Oceanogr.* **30**, 1 (1985)] concluded that the phytoplankton growth rate in the Pacific gyre is 0.2 per day and that all this production is consumed by zooplankton. They concluded that similar steady-state conditions usually exist in other environments, although growth rates are generally higher in coastal areas. This balance between phytoplankton growth and zooplankton grazing does not always exist, since phytoplankton blooms do occur.
17. We thank N. H. Marcus for making zooplankton and phytoplankton cultures available to us for study and for guiding us in their handling. We thank L. Hare and P. Alatalo for assistance in the conduct of the research. Supported by NASA grant NAGW-606 and NSF grant OCE-8416203. Contribution 6165 of the Woods Hole Oceanographic Institution.

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Static and Initiator Protein–Enhanced Bending of DNA at a Replication Origin

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DNA bending has been suggested to play a role in the regulation of gene expression, initiation of DNA replication, DNA packaging, and the recognition of specific DNA sequences by proteins. It has recently been demonstrated that DNA bending can be sequence-directed. Bent DNA has also been observed as a consequence of sequence-specific binding of proteins to DNA. In this report DNA of plasmid pT181 is shown to contain a bend at the replication origin. Furthermore, this bend is enhanced by the binding of the pT181 replication initiator protein, RepC, to the origin.

THE RESULTS FROM RECENT STUDIES have demonstrated the existence of sequence-directed static bends in DNA (*1–5*), as well as bending in response to binding of specific proteins (*1, 6, 7*). A role of DNA bends in such processes as gene expression, initiation of DNA replication, and DNA packaging is yet to be established. However, static bends in DNA may facilitate recognition and binding by specific proteins to these regions, and protein-induced or -enhanced bending of DNA may be critical for providing local structural alterations that are required for DNA transcription, replication, or packaging. DNA containing static bends has been isolated from the kinetoplasts of trypanosomes (K-DNA) (*1, 2*) and the origins of the replication of phage λ (*3*) and simian virus 40 (*4*). Furthermore, it has been suggested that bends in DNA may constitute recognition sites for the replication proteins in the origins of replication of simian virus 40 and λ (*3, 4*). In the R6K plasmid, the binding of the replication initiator protein induces a bend in the DNA at the replication origin (*6*). In addition, DNA bending is enhanced when the λ initiator protein, O, is bound to the origin (*7*). We now demonstrate that DNA from the plasmid pT181 origin of replication contains a bend, and that this bending is enhanced by the binding of the pT181 replication initiator protein, RepC.

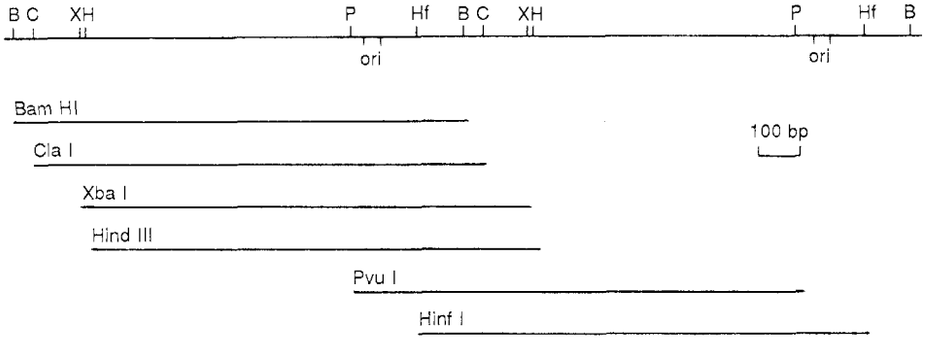

Fig. 1. DNA fragment used for bending analysis. A tandem duplication of the *repC* gene was constructed by partial digestion of plasmid pSK179 (a pUC7 derivative containing the *repC* gene cloned at the Hinc II site) with Bam HI, and inserting the Bam HI fragment from pSK179 that contained the pT181 DNA (*8*). Transformants were isolated, analyzed for orientation of the Bam HI fragment, and plasmid DNA purified by CsCl-ethidium bromide centrifugation. The DNA was digested with restriction enzymes that cut only once within the *repC* gene to generate a series of fragments, each of which was 1075 bp in length. The fragments were purified from acrylamide gels, precipitated with alcohol, and used for migrational analysis. The upper line shows the duplicated DNA with the restriction sites designated as follows: B, Bam HI; C, Cla I; H, Hind III; Hf, Hinf I; P, Pvu I; and X, Xba I. The pT181 origin of replication is designated *ori*. The fragments generated are shown below the restriction map and are designated by the enzyme used to produce them.

We have previously purified the replication initiator protein of pT181, RepC (*8*), and have shown that it binds to a 32–base pair (bp) sequence within the genetically defined origin of replication (*9*). The origin of replication containing the RepC binding site is located within the structural gene for RepC protein (*10, 11*). The protein nicks a single strand of the DNA within the origin and replication probably proceeds by the rolling circle mechanism (*12*). To test for the possibility of a bend in the pT181 origin, we constructed a plasmid that contains a tandem duplication of the *repC* gene (Fig. 1). Permuted restriction fragments, each consisting of 1075 bp, were generated to have the origin of replication located at different sites relative to the end of the fragment (*1*). The location of the origin region varied between 5 and 35% from one end of the fragment. The isolated DNA fragments were incubated in the presence and absence of RepC protein and analyzed by polyacrylamide gel electrophoresis. The series of permuted fragments shows migrational differences in the absence of RepC

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