RAPID RESPONSE PAPER

The abundance and significance of a class of large, transparent organic particles in the ocean

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Abstract—Polysaccharide-specific staining techniques reveal the existence and high abundance of a class of large, discrete, transparent particles in seawater and diatom cultures formed from dissolved exopolymers exuded by phytoplankton and bacteria. Transparent exopolymer particles (TEP), ranged from 28 to 5000 particles m⁻¹ and from 3 to 100s μm in longest dimension at five coastal stations off California. A high percentage of seemingly free-living bacteria (28–68%) were attached to these transparent sheets and films, suggesting that they may alter the distributions and microenvironments of marine microbes in nature. Preliminary coagulation experiments demonstrated that TEP are major agents in the aggregation of diatoms and in the formation of marine snow. The existence of microbial exudates acting as large, discrete particles, rather than as dissolved molecules or as coating on other particles, suggests that the transformation of dissolved organic matter into particulate form in the sea can occur via a rapid abiotic pathway as well as through conventional microbial uptake. The existence of these particles has far reaching implications for food web structure, microbial processes, carbon cycling and particulate flux in the ocean.

INTRODUCTION

Non-living particulate organic matter (POM) is central to microbial processes, carbon cycling, and food web dynamics in the pelagic zone of the ocean. Most of this POM exists as living organisms, detritus, fecal material, exuvia, carcasses, and plankton hardparts that are readily visible using standard techniques of light microscopy (FOWLER and KNauer, 1986). However, we recently observed high rates of particle aggregation in diatom cultures in the laboratory explainable only by the presence of an additional class of large, seemingly invisible, non-living particles. Application of carbohydrate staining techniques to our samples revealed the presence in both natural seawater and diatom cultures of large, discrete, transparent particles, otherwise invisible under the light microscope, apparently formed from polysaccharides exuded by phytoplankton and bacteria. While the existence

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Table 1. Abundance and characteristics of transparent exopolymer particles (TEP)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Depth (m)</th>
<th>Abundance (no. ml⁻¹)</th>
<th>Mean diam. (μm)</th>
<th>Area (mm² ml⁻¹)</th>
<th>% of total cells attached to TEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santa Barbara Channel</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Unfocced diatom bloom*</td>
<td>10</td>
<td>403 ± 46</td>
<td>11 ± 10</td>
<td>0.02</td>
<td>3</td>
</tr>
<tr>
<td>Floccing diatom bloom†</td>
<td>10</td>
<td>179 ± 8</td>
<td>161 ± 222</td>
<td>2.31</td>
<td>90</td>
</tr>
<tr>
<td>Clear water, no bloom‡</td>
<td>10</td>
<td>28 ± 8</td>
<td>11 ± 8</td>
<td>0.001</td>
<td>0</td>
</tr>
<tr>
<td>Monterey Bay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early dinoflagellate bloom§</td>
<td>5</td>
<td>4925 ± 305</td>
<td>8 ± 9</td>
<td>0.25</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>873 ± 168</td>
<td>13 ± 6</td>
<td>0.12</td>
<td>0</td>
</tr>
<tr>
<td>Clear water offshore∥</td>
<td>30</td>
<td>120 ± 8</td>
<td>10 ± 4</td>
<td>0.009</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1427 ± 184</td>
<td>10 ± 9</td>
<td>0.11</td>
<td>0</td>
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<tr>
<td>Diatom cultures</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Chaetoceros gracilis</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Early exponential phase</td>
<td>—</td>
<td>4215 ± 324</td>
<td>4 ± 2</td>
<td>0.05</td>
<td>—</td>
</tr>
<tr>
<td>Stationary phase</td>
<td>—</td>
<td>16,883 ± 323</td>
<td>4 ± 3</td>
<td>0.21</td>
<td>—</td>
</tr>
<tr>
<td>Nitzschia angularis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early exponential</td>
<td>—</td>
<td>814 ± 448</td>
<td>15 ± 13</td>
<td>0.15</td>
<td>—</td>
</tr>
<tr>
<td>Late exponential</td>
<td>—</td>
<td>1122 ± 296</td>
<td>15 ± 18</td>
<td>0.38</td>
<td>—</td>
</tr>
</tbody>
</table>

*34°20'N, 119°50'W, 19 June 1992, unfocced state observed via scuba.
†34°20'N, 119°50'W, 24 June 1992, floccing state observed via scuba. TEP also includes TEP–diatom aggregates abundant at this station.

of transparent marine particles detectable only by staining or electron microscopy has been reported previously (GORDON, 1970; EMERY et al., 1984), these descriptive accounts have been ignored because the abundance, origin, and significance of the particles has remained unknown.

Herein we present data on the sizes, abundances, and origins of this previously disregarded class of non-living particles in the ocean and document its significance as an attachment surface for bacteria and as a major agent in the formation of marine aggregates, including marine snow (aggregates >0.5 mm in diameter).

METHODS

The abundance and size distribution of transparent exopolymer particles, hereafter called TEP, was determined in cultures of the diatoms Chaetoceros gracilis and Nitzschia angularis, and in natural seawater and marine snow samples collected off coastal California (Table 1) by scuba divers (ALLDREDGE, 1991) or with Niskin bottles during June, July, and August 1992. Duplicate 10-ml fresh seawater samples from each station were filtered through 0.4 μm Nuclepore filters, and TEP were made visible by staining the
damp filter for less than 2 s with an aqueous solution of 0.06% acetic acid and 0.02% alcian blue, a stain specific for negatively charged polysaccharides (Parker and Diboll, 1966; Decho, 1990). The particles were then transferred to a slide and the filter removed using the Filter–Transfer–Freeze (FTF) technique (Hewes and Holm-Hansen, 1983).

Control filters dipped in filtered seawater, aspirated to a damp state, and stained contained negligible stained particles. Alcian blue forms a precipitate in the presence of salt. Thus, filter funnels and bases were rinsed with distilled water between consecutive filtrations. We found no difference between the abundances of stained particles on filters rinsed with distilled water prior to staining and filters that were aspirated nearly dry but not rinsed. We also investigated the possibility that freezing caused coagulation of carbohydrate gels accumulated on the filters, producing particles as artifacts. However, microscopic examination of Nuclepore filters containing stained seawater particles that had not been frozen placed on Poretics Corporation Cyto-Clear slides, which render the filter and pores nearly invisible, revealed alcian blue-stained particles of the same type and abundance as did parallel treatments using the FTF technique.

The sizes and abundances of phytoplankton and TEP >3 μm in diameter on each slide were determined using standard light microscopy at 200× magnification. The distribution of bacteria relative to TEP at each station was determined by double staining samples preserved in 0.4% formalin and filtered onto 0.2 μm Nuclepore filters with DAPI (4′,6-diamidino-2-phenylindole) (Porter and Feig, 1980), followed by alcian blue. Although the FTF technique has been reported to yield accurate estimates of bacterial abundance (King and Parker, 1988), we confirmed this by counting total bacterial abundance on parallel treatments using acridine orange staining (Hobbie et al., 1977).

The role of TEP in the formation of marine aggregates was investigated by aggregating cultures of the diatom, Chaetoceros gracilis, and natural seawater samples collected during a diatom bloom off California (34°20′N, 119°50′W) in June 1992, in a laminar shear couvette device in the laboratory (van Duuren, 1968). The 300 ml couvette device consisted of two concentric cylinders separated by a 1 cm gap. A shear of 30 s⁻¹ was used to accelerate coagulation at low natural particle concentrations. Samples were spun for 2 h, and 10 ml subsamples were removed at intervals from the flocculator for alcian blue staining and microscopic enumeration.

The presence of cation bridges in laboratory-made diatom aggregates were determined by placing the flocs in 1 M EDTA (ethylene-diamine tetraacetic acid), a strong chelating agent known to rapidly disrupt cation bridges between adjacent polysaccharide polymers (Decho, 1990).

RESULTS

Particles indiscernible by light microscopy (Fig. 1a) or with a Particle Data El zone Model 180 electronic particle counter (B. E. Logan, personal communication), but made visible after alcian blue staining (Fig. 1b), were present in all natural seawater samples examined. The strong reaction of TEP with alcian blue indicated that these particles contained abundant polysaccharides. TEP ranged from 28 to 5000 particles ml⁻¹ in abundance, and most were from 5 to 50 μm in length (Table 1). Most stainable particles were discrete, highly deformable films, discs or strings, containing no or few visible inclusions (Fig. 1b,h), while others were sheets or ropes of stainable carbohydrates embedded with some detritus (Fig. 1c). Many filmy particles were so thin as to be barely
visible, even with staining. Filtrates of samples passed through 0.4 \( \mu m \) filters contain some TEP that were considerably larger than 0.4 \( \mu m \), indicating that the transparent particles were highly deformable. Smaller TEP sometimes appeared cone-shaped as though it had been trapped in a filter pore while beginning to pass through the filter.

TEP were very abundant in non-axenic cultures of two diatom species, *Chaetoceros gracilis* and *Nitzschia angularis*, especially in late exponential and stationary growth phases (Table 1), reaching concentrations of over 16,000 ml\(^{-1}\). Mean particle sizes ranged from 4 to 15 \( \mu m \). The quantity of TEP in natural seawater varied at several coastal California stations, ranging from 28 particles ml\(^{-1}\) during clear water conditions in the Santa Barbara Channel to 5000 ml\(^{-1}\) during a bloom dominated by dinoflagellates in Monterey Bay. However, because TEP at these stations was relatively small (8–10 \( \mu m \)), the total quantity of TEP, as determined by total two-dimensional surface area, remained relatively small. The surface area of TEP was an order of magnitude higher at a station experiencing an aggregating diatom bloom dominated by species of *Chaetoceros*, *Nitzschia*, *Skeletonema* and *Rhizosolenia* than at any other station. However, abundance of TEP particles at this station was lower (179 ml\(^{-1}\)) because most of the particles were over 100 \( \mu m \) in size (Table 1).

We investigated two of the many possible consequences of TEP abundance in the ocean: its impact on bacterial distributions and its role in aggregate formation. Twenty-four to 68% of the bacteria in our seawater samples were attached to TEP (Table 1), while <5% in all samples appeared attached when only visible particles were examined. Without double staining most bacterial cells would have appeared free-living (Fig. 1g,h).

TEP was also a highly significant component of marine aggregates. Alcian blue-stainable material formed the matrix of all of the natural aggregates of marine snow we examined, including aggregates of diatoms (Fig. 1f), fecal matter, miscellaneous detritus, and even sediment. Aggregates generated in the lab flocculator, both from diatom cultures (Fig. 1d) and natural seawater (Fig. 1e), were also predominantly stainable polysaccharide material. Fewer than 0.1% of aggregates produced in the laboratory flocculator from natural seawater collected during a diatom bloom consisted of diatoms alone. TEP was the predominant component, as estimated by surface area, of all laboratory-made flocs. Laboratory-made diatom aggregates burst apart immediately on placement in 1 M EDTA, indicating the presence of cation bridges binding the subunits of the aggregates.

During artificial aggregation of natural seawater in the laboratory cuvette device, the abundance of TEP and TEP–diatom aggregates decreased over 400% from 623 to 139

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**Fig. 1.** Transparent exopolymer particles (TEP) in seawater and in natural and laboratory-made aggregates. Scale bar is 100 \( \mu m \). (a) TEP are not discernable in 0.02 ml of seawater collected during a diatom bloom dominated by species of *Chaetoceros*, *Nitzschia* and *Skeletonema*. (b) TEP becomes readily visible when another aliquot of the sample from (a) is stained with alcian blue. (c) Sheet-like TEP from a station with no phytoplankton bloom. (d) Flocs formed by aggregation of a late exponential phase culture of *C. gracilis* were composed predominantly of stained polysaccharides rather than cells, evidence of the importance of TEP in aggregate formation. (e) Artificially-produced flocs from the experiment described in Fig. 2 are predominantly TEP but appear densely packed due to the high shear under which they were formed. (f) Discrete polysaccharide particles formed the matrix of natural marine snow collected by scuba divers from a floccing diatom bloom. (g) DAPI-stained bacteria in a natural seawater sample. (h) Alcian blue staining reveals that many of the bacteria in (g) were attached to rope and string-like TEP.
particles ml$^{-1}$ as their mean size increased due to aggregation from 30 to 160 $\mu$m over the 2-h period (Fig. 2). Unaggregated diatom cells, however, only decreased from 73 to 50% of the total cells present, indicating that TEP, rather than diatoms were the major aggregating particles. Unrotated controls showed no change in particle size distributions over time (data not shown).

**DISCUSSION**

**Origin and formation of TEP**

Transparent particles have been observed previously from filtered (Emery et al., 1984) and stained (Gordon, 1970) seawater. Gordon noted that some transparent carbohydrate particles were revealed by the periodic acid-Schiff method, but did not quantify them. Emery et al. (1984) described transparent films associated with diatom debris in the western Pacific using scanning electron microscopy and determined their surface area on slides. However, they believed these films originated from zooplankton feeding webs or bacteria on bubbles. We propose that these earlier descriptions are the same types of particles visualized with our staining technique, although our method reveals the polysaccharide composition and high natural abundances of these transparent particles.

We suggest that TEP originates from the dissolved polysaccharide exudates known to be released copiously by phytoplankton and bacteria (Decho, 1990). Diatoms and bacteria were the only possible sources of organic matter, including TEP, in our cultures. TEP was most abundant in the cultures during stationary phase, the period when diatom exudate production is highest (Mykelstad, 1974; Mykelstad et al., 1989). Moreover, in nature, the quantity of TEP was an order of magnitude higher during flocculation of a diatom bloom than at stations dominated by other types of phytoplankton. However, all types of phytoplankton and bacteria appear to exude dissolved organic molecules, and it is likely that natural TEP is composed of carbohydrate exudates from many sources.
The process by which excreted exopolymers form colloids is well documented in freshwater (Leppard et al., 1977; Massalski and Leppard, 1979). Microfibrils 3–10 nm in diameter and >100 nm in length form when exuded dissolved polysaccharide molecules align via cation bridging. These further coalesce to form mesh-like colloidal particles (Leppard, 1992; Jensen and Søndergaard, 1982). We propose that a similar processes occurs in seawater in the presence of abundant cations. Submicron-sized particles, including colloids, occur in seawater in combined concentrations of $10^{15}$ ml$^{-1}$ (Wells and Goldberg, 1991; Koike et al., 1990). Moreover, over 30% of the "dissolved" organic matter in surface waters can exist in the colloidal size range, much of it as "reactive polysaccharides" (Benner et al., 1992), suggesting that some natural colloidal particles may originate from exudates. We suggest that TEP is formed from dissolved exopolymers by a mechanism similar to that observed in freshwater. Colloids and microfibrils formed of exuded exopolymers further coagulate through processes of aggregation (McCave, 1984) or otherwise coalesce to form the larger particles of TEP we observed. The polysaccharide composition of TEP, the string-like shape of many TEP particles (Fig. 1b, h) that resemble bundles of microfibrils described from freshwater (Leppard, 1992), the high abundance of TEP in exudate-rich diatom cultures and blooms, and the disruption of aggregates by EDTA, indicating the presence of cation bridges, all support this hypothesis.

The actual physical nature of TEP remains to be investigated. TEP appears on filters as two-dimensional sheets and films of discrete particles. In three dimensions, TEP are likely to be highly fractal aggregations of smaller microfibrils and colloids with a high interstitial water content more resembling a gel. However, exopolymer particles are truly particulate in that they are retained on filters, harbor attached bacteria, and are subject to aggregation into larger particles via established coagulation processes. But their physical boundaries with the surrounding seawater may be less clearly defined than more conventional marine particles.

**Significance of TEP**

Our preliminary data indicate that the existence of TEP is highly significant to the chemistry and biology of the pelagic zone. Sheets and ropes of TEP serve as an invisible attachment surface for bacteria and microbes, perhaps altering microbial distributions and providing concentrated substrata for increased metabolic activity. TEP also may provide bacteria with physical refuges from predators unable to feed on surfaces. Marine bacteria may exist in an environment containing webs, strands, and sheets that offer much greater physical structure to the pelagic environment than previously imagined.

The existence of invisible mucus particles is also significant for aggregation processes in the ocean, processes that significantly impact particulate flux and carbon export from the surface ocean (Fowler and Knauer, 1986). Mucus is a well known component of both diatom flocs (Revelante and Gilmartin, 1991; Stachowitsch et al., 1990; Riebesell, 1991) and other types of marine aggregates (Allredge and Silver, 1988), and many studies indicate that biogenic exudates adhere particles together (Decho, 1990; Harris and Mitchell, 1973). However, our coagulation studies demonstrate that mucopolysaccharide exudates participate in aggregation as discrete, independent particles, rather than as sticky coatings on other types of particles. TEP appears to be a major agent in the coagulation of diatoms. The predominance of mucus in marine snow of non-phytoplankton origin (Allredge and Silver, 1988; Biddanda, 1986) and the abundance
of TEP in low-phytoplankton regimes (Table 1) indicates that polysaccharide particles must be significant agents in coagulation under non-bloom conditions as well.

Models based on aggregation theory predict that, except during intense phytoplankton blooms (JACKSON, 1990; HILL, 1992), particle concentrations and collision frequencies are far too low in the ocean for aggregation of particles above submicron sizes to occur at significant rates (McCave, 1984), yet aggregates are abundant in nature. Previous models have not included TEP, the existence of which may help reconcile these models with observation.

The existence of discrete exopolymer particles has significance for many other oceanic phenomena as well. The existence of TEP implies the occurrence of poorly known abiotic transformations between the pools of dissolved and particulate organic matter in the ocean. While bacteria have been assumed to be the major pathway by which dissolved organic matter (DOM) is taken up and transformed to POM, conversion of DOM to POM via abiotic conversion of biotically-produced exopolymers to particulate form also might contribute to otherwise unexplainable high reported turnover rates and large short-term fluxes of DOM (Kirchman et al., 1991). The DOC/POM dichotomy becomes less meaningful as well, given the ability of flexible TEP to squeeze through filter pores. TEP also may provide food particles for zooplankton and protozoan grazers or alternatively, clog zooplankton feeding apparatuses thus inhibiting feeding. Moreover, TEP may provide surfaces for adsorption of a variety of chemical species in seawater. The significance of transparent exopolymer particles awaits much further investigation.

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