The oceanic gel phase: a bridge in the DOM–POM continuum

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Abstract

Recent discoveries reveal that polymer gel particles are abundant and important in the microbial loop, sedimentation processes, biogeochemical cycling, marine carbohydrate chemistry, and particle dynamics in the ocean. The novelty of these discoveries elicited an interdisciplinary discussion among investigators working in marine geochemistry, microbiology, and polymer physics on the significance of gels in the functioning of marine ecosystems. Marine gels are three-dimensional networks of biopolymers imbedded in seawater. They range in size from single macromolecules entwined, forming single-chain colloidal networks, to assembled polymer networks several hundreds of microns or larger. Gels can form in minutes to hours from dissolved organic matter or polymer chains released by phytoplankton or bacteria. They enclose nanoscale microenvironments that exhibit emerging physical, chemical, and biological properties that are drastically different from those of the DOM polymers that make them. Previous studies show that ~10% of surface DOM could be assembled as gels, yielding estimates of $~70 \times 10^{15}$ g of organic carbon. This figure exceeds the global biomass of marine organisms by a factor of 50. The potential huge magnitude of the oceanic gel organic matter (GOM) pool suggests a need to develop reliable quantitative methods to systematically investigate the budget of marine gels and their role in biogeochemical cycling. Gels are particularly important for carbon cycling in that they provide an abiotic mechanism to move organic molecules up the particle size spectrum to sizes capable of sedimentation and eventual sequestration in the deep sea. Macrogels such as transparent exopolymer particles (TEP) are especially significant in sedimentation processes because they appear to be critical for the formation of marine snow and the aggregation of diatom blooms. The discovery of highly abundant gels in seawater also fundamentally changes how we think about the physical nature and microscale structure of the fluid and organic matter field encountered by bacteria, protists, and viruses in the sea. Gels may serve as nutrients and/or attachment surfaces for microbes, as refuges from predation, and as hot spots of high substrate concentration.

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Investigation of gels in the ocean represents an important new area of research ripe for exciting discovery. Areas where future research should be focused include the following: (1) determination of the budgets and pool sizes of gels, (2) investigation of the role of gels in biogeochemical cycling, (3) reconciliation of polymer physics and aggregation theory as explanations for macrogel formation, (4) quantification of the role of gels in sedimentation processes and particle dynamics and, (5) assessment of the role of gels as microhabitats, food sources, and attachment surfaces for marine organisms.
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1. Introduction

The interlinked biological and physical processes by which bioactive elements are cycled in the ocean represent one of the most complex and critical systems on Earth. Although living organisms can act as source and sink of the organic matter in which these elements primarily cycle, the major currency for both their active transfer and long-term storage in the ocean is as small organic molecules in the nonliving pool. This "molecular prerogative" results because the bio-macro-molecules [e.g., polysaccharides (PS) and proteins] composing organisms and tissues must be broken down to subunits (e.g., oligosaccharides and oligopeptides of 5–10 units) of less than about 600 amu (Da) to pass bacterial cell membranes prior to their metabolization. In the ocean, much of this molecular "dismantling" is accomplished by bacterial hydrolytic ectoenzymes acting on the submicron units of dissolved organic substrate. Nutrient elements carried through the microbial loop become available for conversion back to living particulate form either through photosynthesis or via transfer of bacterial production up food webs through protists and zooplankton.

Such biologically mediated cycling between dissolved and particulate organic matter is critical, on a large scale, to the transfer and fate of nutrients because only particles can sink to selectively transport bioactive elements from the lighted surface waters into the ocean's depths. This transport process—the "biological pump"—removes climatically active elements, such as carbon, from direct contact with the atmosphere. Dissolved organic molecules that, for some reason, have been "stranded" between the living and assimilable size extremes (~1–1000 nm; Fig. 1), constitute the most abundant form of biochemicals in the sea, outweighing total living biomass by a factor of ~200 (Hedges and Oades, 1997). Biopolymers can

spontaneously assemble into microscopic polymer gels in seawater (Chin et al., 1998) and play significant roles in the formation of large sedimenting particles (Alldredge et al., 1993; Logan et al., 1995). These findings have fundamentally changed how oceanographers think about processes linking the microbial loop and biological pump in the ocean to the biogeochemical dynamics of the rest of the biosphere and the geosphere (Wells, 1998).

This paper is written by an interdisciplinary team to elicit discussion among marine geochemists, micro-biologists, and geophysicists on the significance of gels in the functioning of marine ecosystems. Because investigations of marine gels are still in their infancy, this paper is not intended as a review, but rather as an interdisciplinary synthesis of concepts and possible research directions.

2. What is a gel?

Hydrogels are made of a three-dimensional polymer network imbedded in water. Water prevents the collapse of the network, and the network entraps water, creating microenvironments that are in thermodynamic equilibrium with the surrounding medium. Gels are a unique form of molecular organization in which the polymer chains that form their networks are interconnected by chemical or physical cross-links, keeping these chains in a statistically stable neighborhood (Tanaka, 1992). The chemical and physical characteristics of the individual polymer chains and the dielectric properties of the water determine the topology and chemical reactivity of the gel network and how it interacts with the entrapped water, smaller organic or metal ion solutes, and living organisms. In general, chemical networks composed of covalently cross-linked polymer chains do not disperse, whereas
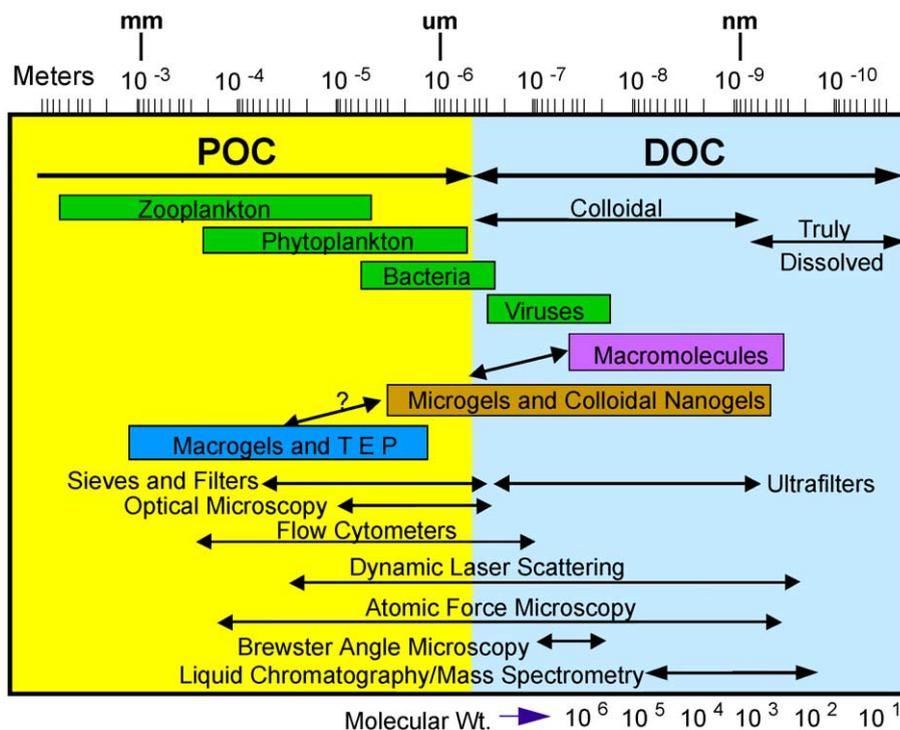
Fig. 1. Size continuum of marine gels.

physical networks stabilized by tangles and hydrophobic, or ionic linkages, can readily disperse (de Gennes and Léger, 1982). This broad range of structural and ultrastructural features gives polymer gels a unique set of emergent physical properties, which constitutes one of the most active and promising areas of research in modern physics (e.g., de Gennes, 1992). They can form in minutes to hours (Fig. 2), to enclose nanoscale microenvironments that exhibit new emerging physical properties, chemical reactivities, and biological availabilities, which are significantly different from those of the dispersed component molecules that make them. Small changes in ambient pH, ionic concentration, or temperature can trigger extensive and abrupt phase transitions in gels, which can radically change their size, density, dielectric properties, chemical reactivities, and permeabilities, and hence, their potential interactions with enzymes and living organisms (Tanaka, 1992).

The characteristic properties of physical networks to continuously assemble and disassemble, incorporating (and releasing) a diverse collection of molecules and ions into a matrix of concomitantly changing volume, porosity, and structure, rank gels among the most heterocomplex systems in nature. The demonstration that gels can assemble spontaneously from DOM polymers remaining in reversible thermodynamic equilibrium with the DOM pool (Chin et al., 1998) has ramifications that might well scale nonlinearly through the microbial loop to the World Ocean and global climate system (Wells, 1998). If, in fact, even a few percent of marine DOM exists as gels (Jensen and Sondergaard, 1982; Stordal et al., 1996; Wen et al., 1997; Santschi and Guo 1997; Chin et al., 1998), then oceanographers will have to completely reconsider the mechanistic underpinnings of such diverse aquatic paradigms as the microbial loop, trace metal scavenging, colloid pumping, humification, and size–reactivity relationships. In a gel-containing ocean hosting dynamic "patchiness" at the nanometer scale, the appropriateness of assigning static bulk properties, such as size, concentration, and age, even comes into question (Azam et al., 1993; Azam, 1998). However, with few exceptions, polymer physics theory and methods have not, as yet, been applied to study the polymer dynamics of seawater DOM. The tremendous
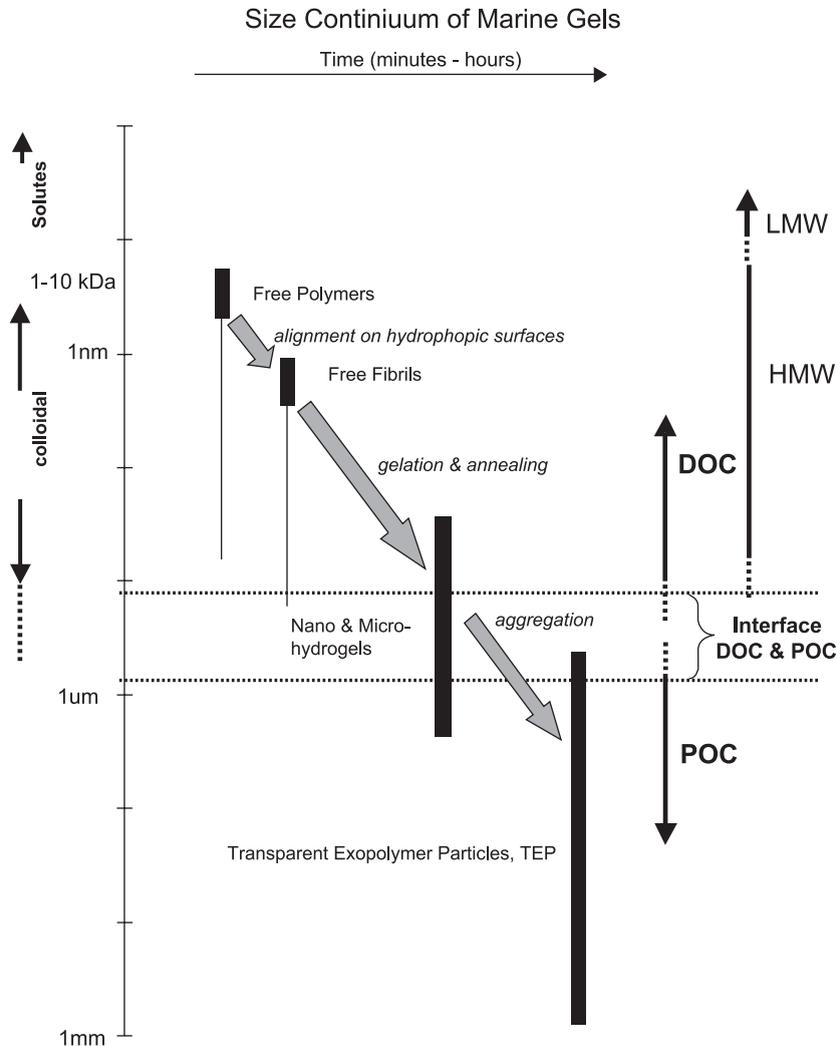
Fig. 2. Methods of investigations for the DOM–POM continuum.

logistical advantage of the gel paradigm for ocean DOM is that the often-nonlinear behavior of these structurally dynamic and heterogeneous organizations can be described and predicted with a rich body of methods and theory drawn from polymer gel physics (Edwards, 1974; 1986).

3. Gels in the organic matter size continuum: from molecules to gigagels

Traditionally, marine organic matter has been divided into a dissolved (DOM) and a particulate (POM) pool, based on the filtration behavior. Organic matter retained on a GF/F, GF/C, or various types of polycarbonate filters with pore widths varying between 0.2 and 1.0 μm is considered POM, whereas the filterable material is considered DOM. Recently, the DOM has been divided into a pool of colloids or submicron particles (Koike et al., 1990; Wells and Goldberg, 1992; Longhurst, 1992; Wells and Goldberg, 1992) and true solutes, or into a low molecular (LMW) and a high molecular weight (HMW) fraction (Kepkay et al., 1993; Benner et al., 1992; Guo et al., 1995). The marine gel phase spans the whole size spectrum from colloids containing single macromole-

cules entwined to form single-chain networks to particles of several 100's μm resulting from the assembly of large number of DOM polymer chains (Figs. 3 and 4). Thus, any division of organic matter based on size is purely operational and reflects only weakly functional features.

3.1. The marine colloidal gel fraction

Because larger organic molecules form more stable self-assembling physical networks, most gel-forming biopolymers are macromolecular colloids (Fig. 1). However, not all colloids form gels. The properties of organic polymer hydrogels are very different (Table 1) from those of "solid" colloids made of aggregated inflexible units (Benedetti et al., 1996; van der Weerd et al., 1999; Wilkinson et al., 1999). The lower limit of ~1 nm for colloids (Fig. 1) corresponds roughly to the minimum size for an organization of nonsolvent molecules that can exhibit contrasting internal and external properties (Gustafsson and Gschwend, 1997). All known and well-characterized polymers released by bacteria and algae are hydrocolloids that can hold large amounts of water, and many of them form gels, often aided by the presence of cations such as K^+ and Ca^{2+}. Anion charge and type of functional group often determine gelation properties and surface activities. For example, kappa and iota carrageenans from red algae, containing one and two sulfate groups per two sugar monomers, respectively, form gels, while the lambda form having three sulfate groups per two

Fig. 3. Marine snow. Contains clear organic matrix, algal fecal pellets and smaller biomolecules (Alldredge and Gotschalk, 1989).

sugar monomers does not (De Ruiter and Rudolph, 1997). Furthermore, carrageenans act as anticoagulants, while alginates from seaweeds, which contain one carboxylic acid group per sugar monomer, act as coagulants (e.g., Libes, 1992). These hydrocolloids usually have double helical structure, or at least helical regions within the molecule, which are held together by Ca^{2+} or H^+ ions, rendering them more rigid (e.g., Kohn, 1972; Rees, 1972, 1977). Aquagenic fibrillar gel-forming microbial exudates are therefore classified as "rigid", while other colloids, such as terrestrially derived (pedogenic) humic acids, are more globular and are called "flexible" polymers (Balnois et al., 1999; Wilkinson et al., 1999), although humic aggregates are held together by metal ions as well (e.g., Buffle and van Leeuwen, 1993; Simpson et al., 2002, 2003a, b). These exopolymeric fibrils are up to several 1000 nm in length, but only 1–3 nm wide, and can exist abundantly attached to cell surfaces or as free colloids (Geesey, 1982; Leppard, 1993, 1995, 1997; Leppard et al., 1997; Santschi et al., 1998). The degree of rigidity of fibrillar biopolymers can be studied using atomic force microscopy (AFM) and characterized by persistence length, end-to-end distance and contour length (Balnois et al., 2000). These measures allow predictions about their physicochemical behavior in solution.

The fraction of free colloidal biopolymers in the ocean is far greater than that found assembled forming gels. The relative magnitude of these two organic carbon pools can be estimated from the relative abundance of DOC and POC, and by considering the close chemical similarity of the two main pools of organic matter in terms of their total and acid polysaccharide (APS) content (below). The number concentration of these free biopolymers remain controversial. Estimates range between ~10^{10} (Koike et al., 1990) and maximal 10^{12} nanoparticles L^{-1} (Wells and Goldberg, 1992). These estimates are of the same order of magnitude as the concentrations of colloids in groundwater from granitic regions or in drinking water (Bundschuh et al., 2001), where the colloid concentration is only a few μg/L (Degueldre et al., 2000). However, size fractionation of oceanic surface water DOM (Guo et al., 1995) showed that typical concentrations of colloidal organic carbon (COC ≤200 nm) in surface ocean environments were as follows: 20–40 μM in the ≥1-kDa (~1 nm), ~11 μM
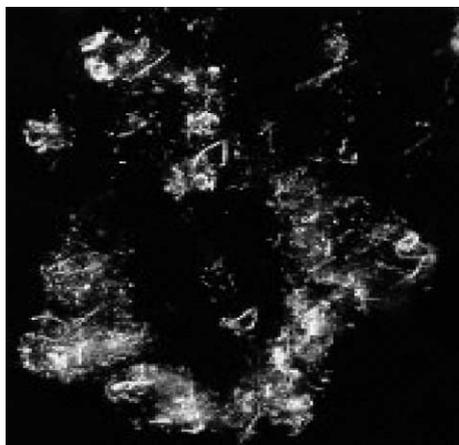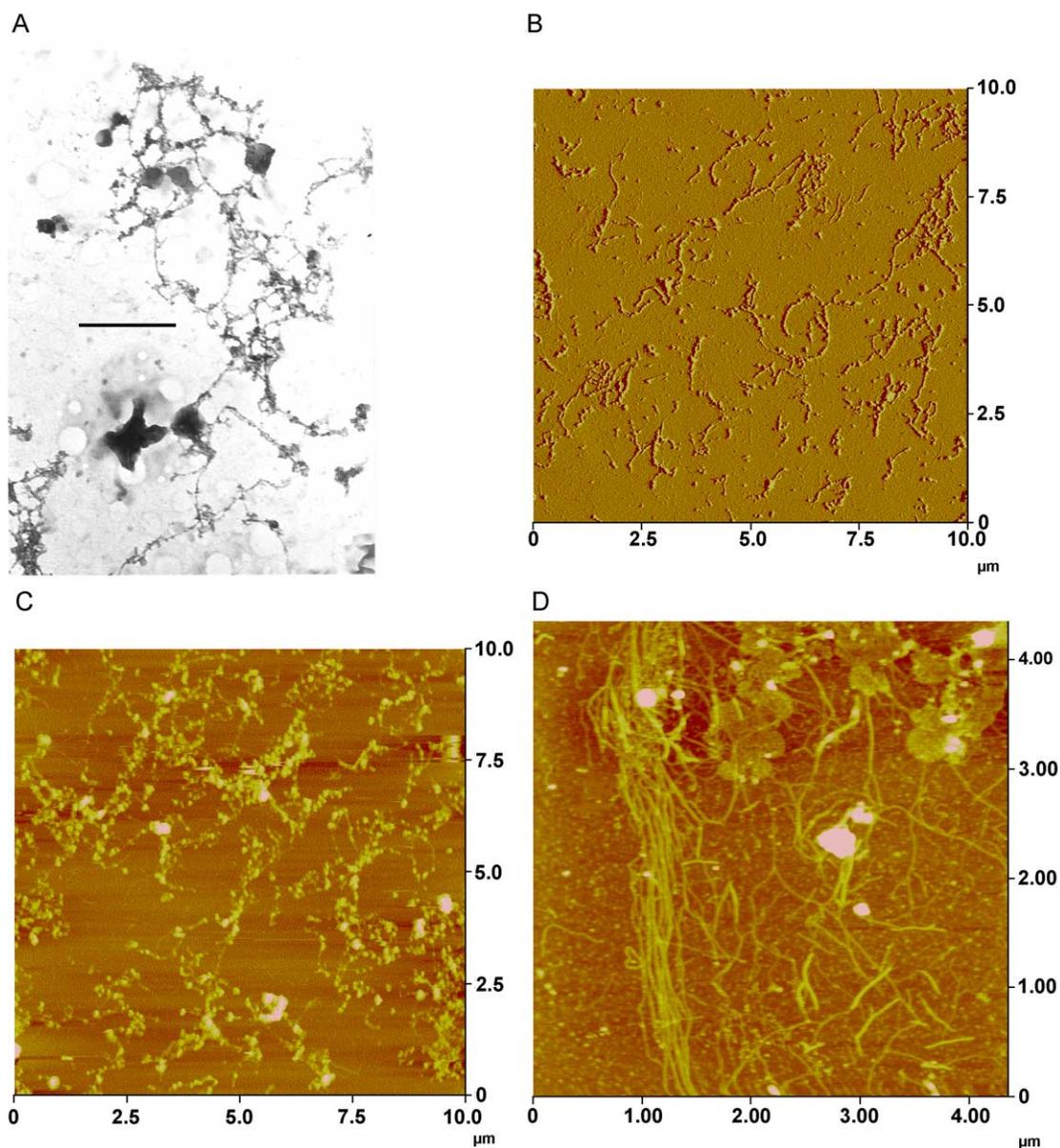
Fig. 4. Fibrillar network of colloidal particles and fibrils, arranged in a pearl-on-necklace fashion (Santschi et al., 1998). (A) Transmission electron microscopy (TEM) of stained fibrillar network, embedded in a hydrophilic resin, which is part of COM (ultrafilter size range of 1–400 nm), sampled from the Middle Atlantic Bight surface waters (courtesy of K. Wilkinson). (B) Atomic force microscopy (AFM) from Middle Atlantic Bight surface water COM; fibrils were also identified by AFM, albeit at lower abundance, in nepheloid layers at 2500 m but not in midwaters. (C) AFM of COM from Galveston Bay waters, TX. (D) AFM image of COM from the surface waters of the Gulf of Mexico, which shows the potential for gel formation of fibrillar macromolecules at the edge of the mica support plate, where the gel-like aggregates likely formed as a consequence of drying.

in the $\geq$3-kDa ($\sim$2 nm), and $\sim$3 μM in the $\geq$ 10-kDa ($\sim$3 nm) size fraction, which allows the estimation of a median molecular weight of 2–3 kDa. From this, an estimated number concentration of colloids in surface seawater of the order of 10^{18} L^{-1} can be calculated [$(6\times10^{23}$ molecules/mol-C)$(2\times10^{-5}$ mol-C/L)].

Table 1
Comparative properties of gels versus solid (none-polymeric) colloids

Characteristics features and reactions	Gels	Other
Structural units bend, fold, reptate and intertwine	Yes	No
Internal dialectric properties different from the bulk	Yes	No
Non-Newtonian rheology and porous structure	Yes	?
Defined internal topology of their polymer network	Yes	?
Defined mechanisms of assembly and stability	Yes	No
Defined kinetics of assembly/dispersion and swelling	Yes	No
Characteristic polymer gel phase transitions	Yes	No
Defined ion (Donnan) and hydrophobic partition properties	Yes	?

These estimates also suggest that a large fraction of unstained colloids remain undetected in studies using transmission electron microscopy (TEM; Wells and Goldberg, 1992). The fraction of colloids assembled forming larger gels is as difficult to assess as that of nanogels (see below).

In a TEM study, Leppard et al. (1997) observed a wide range of colloidal particle types, dominated by 1–60 nm "fractal aggregates". Santschi et al. (1998) used TEM and AFM to image colloidal organic matter (COM) components down to a fraction of 1 nm. In the midst of 10^3-year old colloidal organic matter, polysaccharide-rich fibrils of modern origin (based on ^{14}C chronology) were found abundantly across the colloidal size range in the euphotic zone of marine environments (Santschi et al., 1998). These fibrils coexisted with a low concentration of spherical bodies similar to Koike particles, as well as the fractal aggregates of Leppard et al. (1997). A generic relationship between such submicron aggregates and larger marine snow composites (e.g., Alldredge and Silver, 1988; Alldredge and Jackson, 1995; Jackson and Burd, 1998) rich in transparent exocellular polysaccharides (Zhou et al., 1998; Mari and Burd, 1998; Passow, 2000) has been suggested (Leppard et al., 1996a, b).

While polysaccharidic fibrils, as a testimony of the presence of the gel phase, are abundant in the surface ocean, they appear absent from middepth regions and reappear again in nepheloid layers of the deep ocean, as has been documented by Santschi et al. (1998) in AFM images of COM.

3.2. Marine microgels

Dynamic laser scattering reveals that polymeric DOM in 200-nm filtered surface seawater, from latitudes ranging from the Arctic Ocean to the Gulf of Mexico, can spontaneously assemble following a second-order kinetics (Chin et al., 1998). Brewster angle microscopy corroborates a two-step kinetics, as it reveals that free polymeric DOM probably reaches critical concentration at the air–water interface forming rafts that collapse, forming nanogels that then diffuse into the bulk water, annealing with each other to form microgels. The resulting microgels contain polysaccharides, proteins, and nucleic acids chains, forming noncovalently cross-linked physical polymer networks with random tangled topology stabilized by Ca ion bonds. Because of their loosely weaved topology, these gels remain in reversible assembly/dispersion equilibrium. They spontaneously assemble, but they can readily disassemble and disperse in the absence of Ca ionic bonds. In response to changes in pH and temperature, they can undergo characteristic polymer gel phase transition to a condensed phase, drastically collapsing and increasing their sedimentation coefficient (Chin et al., 1998).

Marine microgels probably represent a substantial pool of bioactive substrate on Earth. The thermodynamic yield of DOM assembly/dispersion indicates that, at equilibrium, ~10% of surface seawater DOM could be self-assembled, forming microgels (Stordal et al. 1996, Guo and Santshi, 1997, Chin et al., 1998). If this yield reflects even approximately the assembly properties of the global ocean DOM pool (700×10^{15} gC), then $\sim 70 \times 10^{15}$ g of organic carbon may occur as microgels in the ocean. This mass exceeds the global biomass of marine organisms by a factor of 50. Within the magnitudes of "size-related bioreactivity" of DOC (Kirchman et al. 1991; Amon and Benner 1994; Santschi et al., 1995), this enormous mass of micro- and nanogels is among the largest dimensional domain and represents a huge pool of biodegradable organic carbon accessible to bacteria. Although the size distribution of seawater organic matter is skewed toward smaller components, the colloidal fraction

makes up ~25% of total DOC (Benner, 2002; Benner et al., 1992). Thus, the ocean probably contains >150 Gt of DOC in the colloidal size range, where the potential of the component macromolecules to form gels is greatest (Edwards, 1974). This huge magnitude of the oceanic micro- and nanogel pools points to an urgent need to develop reliable quantitative methods to systematically investigate the budget of marine gels and their role in biogeochemical cycling. In this regard, the application of flow cytometry, fluorescence spectroscopy, and dynamic laser scattering offers great potential for the detection and quantitative assessment of microgels concentration in seawater.

3.3. Macrogels—transparent exopolymer particles (TEP)

Nanogels and microgels can eventually grow further by continued collision and annealing (Wells and Goldberg, 1994; Kepkay, 1994), forming larger macrogels and gel-like particles, such as transparent exopolymeric particles (Alldredge et al., 1993; Passow, 2002a, b), that are truly POM. Several species of phytoplankton can readily release assembled gel material that, during blooms, can reach gigantic gigagel (m) dimensions (e.g., Mecozzi et al., 2001; Ciglenecki et al., 2000). TEP are especially significant in sedimentation processes and carbon cycling in the sea because they form the matrix or "glue" of aggregates of particulate matter and appear to be critical for the formation of marine snow and the aggregation of diatom blooms (Alldredge et al., 1993; Logan et al., 1995; Passow, 2002 a,b). Because TEP are formed directly from DOC, they represent a potentially highly significant pathway by which DOC can be transformed into POC and sequestered via sedimentation.

TEP, operationally defined as Alcian Blue stainable particles retained on filters (Alldredge et al., 1993), have, in the past, often been called mucus- or gel-particles. Several lines of circumstantial evidence suggest that TEP qualify as gels: (1) ubiquitous presence of acidic polysaccharides in TEP; (2) the likely constituents of TEP (carrageenans, alginic acid, and xanthan) are known to form gels; (3) TEP can pass 0.4- or 0.2-μm filters (Passow and Alldredge 1995); (4) TEP spontaneously form from dissolved fibrillar colloids (Passow 2000); and (5) TEP almost instantaneously break up in the presence of Ca

chelators, such as EDTA (Alldredge et al., 1993). But the nature of their association, e.g., whether they consist of networks which undergo phase transition, has not been shown, and thus, proof of their gel characteristics is still missing. Even less is known regarding possible gel characteristics of the proteinaceous Coomassie stained particles (CSP; Long and Azam, 1996), or other potential candidates for micro- and macrogels (Kerner et al., 2003).

The number concentrations of TEP >5 μm vary between 10^3 and 8×10^6 L^{-1} and those of TEP >2 μm between 3×10^6 and 4×10^7 L^{-1} (Passow, 2002). Acidic groups that are stained with Alcian Blue, albeit at different intensities, include carboxyl, sulfate, and phosphate (Passow, 2002a,b; Hung et al., 2003a,b). TEP harvested from four different environmental situations in seawater was enriched in sulfate-rich desoxy sugars (fucose and rhamnose) and, to a lesser extent, galactose, but concentrations of uronic acids (URA) were small, suggesting that sulfate ester groups were responsible for the acidic properties of these TEP particles (Mopper et al., 1995; Zhou et al., 1998). However, the URA and sulfate content of exopolymeric polysaccharides (EPS) from even a single planktonic species, such as cyanobacteria, can be highly variable (De Philippis and Vincenzini, 1998). The high but varying C/N ratio of TEP (Engel and Passow, 2001; Mari et al., 2001) suggests that, although nitrogen-rich material can be associated with TEP, these substances are minor components.

The total carbon content of TEP, estimated by determining directly the carbon content of isolated TEP, using xanthan as standard (Mari, 1999; Engel and Passow, 2001), is in the range as for phytoplankton, e.g., 10 to 800 μg carbon L^{-1} seawater. The carbon content measured by this method includes the total amount of carbon associated with Alcian-Blue-stained particles, including acidic and associated neutral sugar units, lipids, glycoproteins, etc. The content of the acid polysaccharides (APS) in TEP, relative to POC, was estimated by Hung et al. (2003a) by calibrating the Alcian Blue method with alginic acid, which has an anion density of almost one carboxyl group per sugar monomer. In the Gulf of Mexico, they found the total APS concentration of size fractionated POC to be 0.02–0.2 μM-C. The majority of APS was in the 0.7–10 μm fraction and generally decreased with increase in particle size. APS

was 9–19% of total polysaccharides, but ≤3% of POC. Uronic acids (URA) made up 20–30% of APS.

3.4. Formation of micro- and macrogels

The abiotic formation of gel-like marine particles on bubbles was observed over 40 years ago (Riley, 1963; Sutcliffe et al., 1963; Johnson and Cooke, 1980). Mucus particles were first described for freshwaters in 1977 (Leppard et al., 1977), and for marine systems, in 1984 (Emery et al., 1984). The ubiquitous presence of mucus flakes in the ocean was postulated shortly thereafter (Smetacek and Pollehne, 1986). Aggregates of mucilage or mucus macromolecules can reach millimeters to centimeters (marine snow: Fig. 3) to meters in size (e.g., Adriatic Sea: Herndl and Peduzzi, 1988). All mucilages swell to produce gels or highly viscous solutions when dispersed in water, thus the term "slime". Gel layers are also naturally part of the outer layers of aquatic microorganisms (e.g., Leppard et al., 1977; Decho, 1990; Leppard, 1992, 1993), which are also called EPS, which stands for exopolymeric particles or exopolysaccharides, depending on the author. The importance of the spontaneous formation of gel particles in the ocean has, nevertheless, only emerged within the last decade (Alldredge et al., 1993; Chin et al., 1998; Passow, 2002a, b), as new methods to determine gels in their different phases have resulted in a flood of research activity.

Two major hypotheses have been proposed for the formation of micro- and macro-gels: aggregation of polymer fibrils and colloids by collision to form larger particles, and spontaneous assembly of DOM polymers to form larger polymer gels. Both mechanisms are supported by an extensive theoretical and empirical literature developed in fields outside of marine science. A quantitative understanding of the extent to which these two processes occur, overlap, and complement each other in the formation of marine gels in the nature and the size ranges and particle types where each dominates is critical if we are to develop models that accurately predict particle dynamics, the fate of DOM, and biogeochemical cycles in the ocean.

The particle coagulation theory was originally developed by aerosol physicists (see Twomey, 1977) and has been applied to explain particle size spectra in natural waters (see O'Melia, 1972; McCave, 1984; Jackson, 1990). An aggregate is a particle formed by the collision and subsequent sticking of two smaller particles. Larger and larger aggregates are formed by repetitive collision and coalescence. The coagulation theory quantitatively describes the rate of change in particle abundances and size distributions generated by this repetitive collision and sticking process. According to this theory, aggregation rate is a function of the sizes, concentrations, and stickiness of colliding particles and the intensity of the physical processes colliding them together. For colloidal-sized particles, these physical processes include Brownian motion, shear (both laminar and turbulent), differential settlement, surface coagulation, diffusive capture, filtration, and, for living particles, motility (Kepkay, 1994). Brownian motion is the dominant collision mechanism for submicron particles in still water, such as the deep ocean (McCave, 1984; Honeyman and Santschi, 1989; Jackson, 1990; Jackson and Burd, 1998).

According to the polymer gel theory, the polymer networks that form the matrix of polymer gels result from spontaneous or induced assembly of polymer chains (Doi and Edwards, 1984). Assembly is not explained by collision or by a stickiness argument but occurs when the interchain distance allows polymers to interact with each other via chemical (covalent) or physical bonds (entanglements, electrostatic, and hydrogen bonding, or hydrophobic/hydrophilic interactions, van der Waals forces, etc.), which results in the formation of cross-links. In polyelectrolyte networks, assembly depends on the pH, ionic composition, and dielectric properties of the solvent that scale the extent of the Debye field of interaction among polymer chains, and the concentration, as well as the physicochemical features, of the polymer chains and their corresponding second virial coefficient, i.e., the presence of functional reactive groups in the case of chemical networks, or, in the case of physical networks, the chain size and dynamic topology (linear or branched structure, Kuhn segment length, and Flory's rubber elasticity), presence of hydrophilic/hydrophobic domains, polyelectrolyte properties, and charge density, etc.

The topology of assembled networks can range from random to semiordered (nematic liquid crystalline) to truly ordered architectures (Viney et al., 1993). However, the physical properties of polymer gels depend primarily upon the nature and degree of cross-

linking. In chemically cross-linked networks, polymers are covalently interlinked, forming gels that are stable and can reach large dimensions. The mobility of polymer chains is constrained to local diffusion within the free path length between cross-links. However, in physical networks stabilized by tangles and low-energy interactions, polymers can readily walk out of the matrix and disperse. In this case, an additional degree of freedom to undergo axial random walk (reptational diffusion) implies that the stability and the equilibrium size of physical gels depend on the second power of polymer chain length (de Gennes and Léger, 1982). An additional influence in the stability of physical networks is that it takes a critical number of cross-links to be broken before chains can disperse. Because cross-links are continuously making and breaking, the probability that a critical number of cross-links break simultaneously decreases with the total number of cross-links holding a polymer. These simple physical principles can explain why native marine gels (and associated metals) assembled from large polyionic exopolymer chains can reach macroscopic dimensions (Passow, 2000; Stordal et al., 1996; Wen et al., 1997), while tangled networks assembled from smaller 200 nm filtered DOM polymers form microgels that do not grow beyond a few microns size (Chin et al., 1998). This behavior results from the fact that longer chains can be held together by a larger number of low-energy cross-links (ionic, hydrophobic, tangles, etc.). Although short- chain organic molecules can also form networks, these grow to smaller size and are short lived because it takes fewer bonds broken to get polymer chains free. At the limit, chain residues resulting from extensive fragmentation, like in UV photolysis or biochemical cleavage, fail to self-assemble and remain in the solution (Chin et al. 1998; Kerner et al. 2003; Orellana and Verdugo 2003; Orellana et al. 2000). An interesting prospect is that this hydrolyzed DOM polymer fraction, which is too large to be transported across the bacterial wall, yet too small to assemble, may form a truly refractory residue of fossil polymers.

4. Gels as a fraction of the organic pool

Because substances belonging to the gel phase are rich in acid polysaccharides, one can gain some

information on the relative upper limit of the abundance of the gel phase in the ocean by reviewing the chemical composition of POM and DOM. Available evidence shows that polysaccharides in the ocean water are mainly composed of neutral polysaccharides, with identifiable acidic compounds usually making up only a minor fraction (Aluwihare et al., 1997, 2002; Repeta et al., 2002; Santschi et al., 2003; Hung et al., 2003a,b). Results of determinations of total carbohydrate (TCHO), neutral polysaccharides (PS), acyl PS, acid PS (APS), and URA abundance in POM and DOM from different oceanic environments are given in Tables 2 and 3. The difference between acyl and acid PS is that acyl polysaccharides, determined by ^{13}C and ^{1}H NMR, contain all carboxylated groups, including those esterized or bound to amino groups, while the term acid PS or APS refers to only those acidic groups that are free.

Given the relative pool sizes of the different POC fractions, the following relationships between organic carbon fractions should hold:

$$[POC] > [TCHO] > [neutral\ PS] > [acyl\ PS] > [APS] > [URA] \tag{1}$$

Estimates of APS in POC and DOC, listed in Tables 2 and 3, are in good agreement with this mass balance argument (Eq. (1)), with, on average, [TCHO]/[POC] of 9–24%, [APS]/[POC] of 1–3%, and [URA]/[POC] of 0.2–0.9%. The relatively low abundance of PS compounds agrees with results by Bergamaschi et al. (1999) and Hamanaka et al. (2002), who also showed that most of the sinking particulate matter from oceanic environments was composed of neutral polysaccharides, with total GC-MS quantifiable URA making up only 0.3–1.2%. Similarly, Walters and Hedges (1988) reported that only 1.8% of planktonic C is composed of URA. While arguing that the largest fraction of marine DOM is composed of acyl polysaccharidic biopolymers, Aluwihare et al. (2002) and Repeta et al. (2002) report that most of the carbohydrates in the colloidal macromolecular fraction are also composed of neutral polysaccharides, with identifiable URA compounds (glucuronic and galacturonic acid) making up only about 2–5% of the total carbohydrate content, and the total acyl group carbon amounting to not more than

Table 2

Concentrations of marine dissolved carbohydrates (TCHO), polysaccharides (PCHO), monosaccharides (MCHO), aldose, and uronic acids (URA) normalized to DOC in dissolved and colloidal samples

Location sample type	TCHO (C %)	PCHO (C %)	MCHO (C %)	Aldoses (C %)	URA (C %)	Reference and analytical method
Pacific (S,C)	20–30					1, MBTH and NMR
Pacific (S)	25±10	19±12	7±4			2, MBTH
Gulf of Mexico (S)	21±6	14±9	7±4			2, MBTH
Atlantic (S)	15±12	9±2	6±1			2, MBTH
Gerlache Strait (S)	16±2	13±2	3±2			2, MBTH
Mesocosm (S)	20–50			8–20	<5	3, MBTH and HPLC
N. Pacific (C, 10m)				31		4, GCMS
Sargasso Sea (C, 25m)				25		4, GCMS
GOM (C, 25m)				25		4, GCMS
Pacific (S)				2–4		5, HPLC
Estuaries (S)	11			4		6, MBTH and HPLC
Open ocean (S)	19			10		6, MBTH and HPLC
Fjord (Norway)	15–21					7, TPTZ
Pacific (C)				15–20		8, GCMS
Peru (C)				19		9, GCMS
Delaware Estuary (S)	9±3	5±3	4±2	2.6		10, TPTZ; 10a, HPLC
Pacific, Atlantic (C)				21±6[a]		11, HPLC
Galveston Bay (C)	11–23				10	12, TPTZ and Spec.
Galveston Bay (S)	13–24				1	12, TPTZ and Spec.
Gulf of Mexico (S)						13, TPTZ and GCMS
Middle Atlantic Bight (C)	11–41					14, TPTZ and Spec.
N. Pacific and Bering Sea (S)					11–43[b]	15, Spec.

Abbreviations—S: seawater; C: colloid (>1 kDa); MBTH: carbohydrate was measured by the colorimetric detection with MBTH (3-methyl-2-benzothiazolinone hydrazone hydrochloride); and TPTZ: carbohydrate was measured by the colorimetric detection with TPTZ (2,4,6-tripyridyl-*s*-triazine).

References—1: Benner et al., 1992; 2: Pakulski and Benner, 1994; 3: Mopper et al., 1995; 4: McCarthy et al., 1996; 5: Skoog and Benner, 1997; 6: Borch and Kirchman, 1997; 7: Borsheim et al., 1999; 8: Aluwihare et al., 2002; 9: Repeta et al., 2002; 10: Witter and Luther III, 2002; 10a: Kirchman and Borch 2003; 11: Benner and Kaiser, 2003; 12: Hung et al., 2001; 13: Hung et al., 2003a; 14: Santschi et al., 1998; 15: Sakugawa and Handa, 1985.

[a] The sum of neutral (aldoses) and amino sugars.

[b] Percent of TCHO.

10% of the total organic carbon content, despite the fact that the acyl-C-containing biopolymer makes up a major fraction of the total.

Another constraint on the quantitative importance of acidic sugar compounds comes from the size continuum model of Amon and Benner (1994) and the supporting radiocarbon data of Santschi et al. (1995), which demonstrate that radiocarbon ages of different COM size fractions from the surface ocean increase with decreasing colloid size. Both groups of authors show that the carbon flow in the surface ocean is from high to low molecular weight. If surface-active APS containing biopolymers would make up a large fraction of POC, then the main OC flux would be from small to large molecules and particles (via coagulation). While significant amounts of organic matter were observed to be transferred up the size spectrum through bacteria grazing by protists, followed by liposome or "picopellet" excretion (e.g., Nagata and Kirchman, 1999), the relative importance of microbial degradation vs. grazing loops is still open to question.

Santschi et al. (1998) reported that the 100% polysaccharide-enriched fraction of COM, containing pure fibrils, has a modern radiocarbon age ($\Delta^{14}C=26‰$), while the bulk COC from surface waters is 750 to 1750 years old ($\Delta^{14}C=-112$ to $-195‰$). From a mass balance perspective, assuming that the nonfibrillar matter has a $\Delta^{14}C$ value equal to that of whole DOC of $-155‰$ (Bauer, 2002), these $\Delta^{14}C$ data would allow for, at most, 30% of fibrillar polysaccharide-rich TEP-precursor

Table 3
Concentrations of marine particulate carbohydrates (TCHO), monosaccharides (MCHO or aldoses), and uronic acids (URA) normalized to POC in different marine organic samples

Sample type	TCHO (C %)	MCHO (C %)	URA (C %)	Reference and analytical method
Sediment	11.9	10.6	1.3	1, GCMS
Plankton	7.4	5.6	1.8	1, GCMS
Phytoplankton	37	15		2, MBTH
Cultured Diatom	5–8	5–8		3, GCMS
Marine POM		4–13		4, HPLC
Fresh POM	16.3		2.3[a]	5, GCFID
Sinking POM	13.8		0.7[a]	5, GCFID
Plankton		2–17		6, GCMS
Floating traps		5–18		6, GCMS
Moored traps		11–17		6, GCMS
Phytoplankton	18–45			7, MBTH
Phytoplankton		6.9	0.5	8, GCMS
Zooplankton		5.8	0.2	8, GCMS
Sediment traps	7–25	6–24	0.3–1.2	8, GCMS
Exopolymers			<5	11, HPLC
Marine POM	9–24		0.2–0.9	12, TPTZ and Spe. and GCMS
Sinking POM	6–13		0.6–1.1	12, TPTZ and Spe. and GCMS

Abbreviations—MBTH: carbohydrate was measured by the colorimetric detection with MBTH (3-methyl-2-benzothiazolinone hydrazone hydrochloride). TEP: transparent exopolmeric particles. TPTZ: carbohydrate was measured by the colorimetric detection with TPTZ (2,4,6-tripyridyl-*s*-triazine). ABS: Alcian Blue stain.

Bulk carbohydrate concentrations were measured by spectrophotometric analysis or HPLC method (e.g., the summation of five to nine neutral monosaccharides). Monosaccharide concentrations were measured by HPLC method.

Spe.: uronic acids were measured by spectrophotometric analysis.

References—1:Walters and Hedges, 1988; 2: Biersmith and Benner, 1998; 3: Cowie and Hedges, 1996; 4: Skoog and Benner, 1997; 5: Hamanaka et al., 2002; 6: Hernes et al., 1996; 7: Biddanda and Benner, 1997; 8: Bergamaschi et al., 1999; 11: Mopper et al., 1995; 12: Hung et al., 2003a.

[a] Surface-active polysaccharides extracted by methanol.

biopolymers of modern age in bulk COM from the surface ocean. Although fibrils are the most noticeable component of COM (Fig. 4; Santschi et al., 1998), this assessment is reasonable because the fibrils are mostly covered with smaller polymers like pearls on a necklace, with the majority of the mass being in the pearls rather than in the string (Santschi et al., 1998).

Such an assessment of lower abundance of APS compounds does not diminish their importance for oceanographic processes. On the contrary, the importance of TEP-like biopolymers in controlling the scavenging of metals and radionuclides and the coagulation/flocculation of particles (Guo et al., 2002; Santschi et al., 2003), as well as in the early development of biofilms, is largely due to their surface-active nature. They can also initiate or modify precipitation of MnO_2 and FeOiOH (Cowen and Bruland, 1985), SiO_2 (Kinrade and Knight, 2002), and $CaCO_3$ (Leveille et al., 2000). Moreover, acid polysaccharide-rich particles function in the extracellular milieu by forming not only flocs (Alldredge et al., 1993; Mopper et al., 1995) and biofilms (Leppard, 1997), but also by binding extracellular enzymes in their active forms, scavenging trace metals and Th(IV), as well as other radionuclides from the water (Quigley et al., 2001, 2002; Guo et al., 2002; Santschi et al., 2003), immobilizing toxic substances, altering the surface characteristics of suspended particles, and modifying the solubility of associated molecules (Costerton, 1984; Leppard, 1997).

5. Microbial interactions with gels

The discovery of highly abundant gels decorating an organic matter continuum in seawater fundamentally changes how we think about the physical nature and microscale structure of the organic matter field encountered by bacteria, protists, and viruses in the ocean. The implications of the new view are profound for organic matter cycling, microbial food web structure, as well as the microbes' ecology. However, before discussing some of the possible implications, it is logical to ask whether the microbes display adaptations, in situ, for response to the structure and dynamics of the microscale field of organic matter (or whether they "ignore" the gels).

The most direct evidence for bacterial response to gels comes from Alldredge et al. (1993), who differentially stained seawater samples for TEP and bacteria and found a large fraction (42%) of bacteria to be attached to TEP. Long and Azam (1996) demonstrated Coomassie stainable particles (CSP) to be colonized by bacteria, although the degree of colonization was not quantified. Bacterial behavior in live seawater

samples, examined by dark-field microscopy, is also consistent with adaptations for interaction with organic matter hot spots, presumably including gels. Blackburn et al. (1998) showed active clustering of bacteria around a killed ciliate, while Grossart et al. (2001) demonstrated motility to be a common phenotype in natural bacterial assemblages. Furthermore, there is anecdotal dark-field microscopy evidence of clustering of bacteria around "invisible" loci in seawater samples, possibly gel particles. However, due to the very nature of transparent particles, it has not been possible to microscopically examine whether bacteria respond behaviorally to TEP and CSP (stains were not used because they might change bacterial behavior towards gels). In any event, there appears to be ample evidence consistent with the hypothesis (and expectation, based on the knowledge of bacterial survival strategies) that pelagic bacteria can respond to the presence of gels in seawater.

Gels may serve as nutrients and/or attachment surfaces and offer hot spots of high substrate concentrations. The gel substrate can be rendered accessible to bacterial permeases through polymer hydrolyses by ectoenzymes, such as proteases, glycosidases, lipases, and nucleases. The consequences of gel–bacteria interaction will thus depend not only on increased substrate concentration found in the gel matrix but also on the matching of polymer composition and the type and level of bacterial ectoenzymes being expressed. Simultaneous expression of high- and low-flow monomer permeases in marine bacteria (Nissen et al., 1984) could enable tight hydrolysis–uptake coupling. However, the hydrolases would also tend to disassemble the gels by breaking the constituent polymers. Endohydrolases (causing breaks within the polymer chain) would disassemble the gel more efficiently than exohydrolases (causing sequential removal of monomers from termini) for a given rate of bond cleavage. This raises the intriguing possibility that bacteria have evolved strategies for hydrolytic attack on gels that optimally balances the rate of monomer acquisition with the time frame of gel persistence as a hot spot. Too rapid a disassembly would disperse and dilute the constituent organic matter. An additional kinetic challenge for bacteria is that gel utilization would be abruptly terminated if a protist ingests the gel and its associated bacteria (Sherr,

1988). In any event, bacterial hydrolytic enzymes are likely to be critical variables in bacteria–gel interactions, as well as in gel behavior and stability in seawater.

Most bacterial hydrolases are cell-surface bound, thus, their action on gels is spatially defined and explicit. However, a small but variable fraction of hydrolase activity is often found dissolved, and this fraction tends to increase during the death phase of phytoplankton blooms. Dissolved hydrolases may be derived from sources such as bacteria, lysing phytoplankton, protists grazing activity, and metazoan feces. However, there is no study addressing the relative inputs of the various sources or the stability of the released enzymes in seawater. Although the activities of dissolved hydrolases are typically low, they could influence the behavior and stability of the gels in a more general spatial context because of their diffusibility. Thus, dissolved hydrolase activities might also be a significant variable in marine gel dynamics.

In addition to their role as nutrient hot spots for bacteria, the gels may provide surfaces for bacteria attachment and interactions. One might envision the development of specific species or consortia on individual gel particles. The ecological and biogeochemical implications of this role of the gels are profound. The microhabitats offered by the great abundance and chemical variety of gels could contribute to the maintenance of high microbial biodiversity in the pelagic ocean. This possibility is consistent with the finding (Long and Azam, 2001) of variation in bacterial species composition at the millimeter scale in seawater. The maintenance of high biodiversity on gels has important implications for organic matter transformation and decomposition; it would increase the diversity of biochemical transformations mediated by the microbial assemblage acting on the organic matter pool in the ocean.

Much more work is needed to explore microbial diversity at the micron scale. We know more about some of the heterotrophic bacteria colonizing large particulate detritus (100 μm to cm; e.g., marine snow) that probably includes marine gels. The microbes inhabiting these detrital particles differ from those living in the bulk water (Delong et al. 1993; Crump et al., 1999). Bacteria in the *Cytophaga–Flavobacterium* cluster are often the most abundant ones in marine

snow and other large detrital particles (reviewed by Kirchman 2002). The success of the *Cytophaga*-like bacteria on particulate detritus is probably due to their capacity to utilize biopolymers (Cottrell and Kirchman 2000), although many cultured representatives are able to glide across surfaces, which may be adaptive for microbes colonizing detritus. Furthermore, the high abundance of apparently free-living *Cytophaga*-like bacteria may actually be due to their association with microgels that are invisible by standard staining methods (e.g., DAPI and probing by fluorescence in situ hybridization). The well-documented differences in microbial community structure between "free-living" bacterial communities and large particulate detritus suggest that gels of all sorts have a large impact on microbial diversity in the oceans.

6. Major directions for future research

6.1. Determination of budgets and pool sizes: reconciling different methods

Currently, no unifying method exists that can quantify colloidal gel-polymers in the ocean. The kinetics of nano- and microgel formation have been measured using dynamic laser scattering spectroscopy, and the thermodynamic yield of microgel assembly can be readily assessed by flow cytometry (Chin et al., 1998). The Alcian Blue method is, although very useful for depicting the functional properties of TEP, only semiquantitative, regardless of the standards that are used. Even for TEP, which are the most thoroughly investigated group of gel particles, the relative chemical composition is still unknown (Passow, 2002a,b; Hung et al., 2003a,b). In addition, extraction and purification methods of gel-forming fibrillar TEP precursor molecules for the purpose of chemical and instrumental characterization, as well as physicochemical experimentation (e.g., investigation of their gel forming properties), will require increased scrutiny and attention because compositional results are extraction methodology dependent. Methodological difficulties plague the correct assessment not only of TEP but also of POC and DOC. The existence of TEP precursor molecules in the DOC fraction is likely also the

cause of methodological difficulties of assessing POC correctly (Gardner et al., 2003). A foremost problem is the assumption that particles, mostly gels, and dissolved DOM biopolymers act independently during the filtration process. In fact, gels retained by filters can act as porous plugs in the filter. These plugs, with their own pore size and their own affinities, can selectively retain unknown amounts of free DOM moieties, which, once hold on to the gel network, become functionally classified as POM. Fibrils 0.4 to 4 μm long and 1–3 nm thick can readily pass a 0.4-μm filter (Santschi et al., 1998), but a fraction might, depending on conditions, be retained on the filter. Conversely, the original fraction of the native gel, once retained by the filter, will appear drastically enriched by molecules that, in their native state, were "dissolved" polymers. Alternatively, high flow filtration could take apart polymer networks that, in their native state in seawater, were part of gel matrix. These potential artifacts require thorough verification of current protocols, and new methods are urgently needed to investigate the kinetics and thermodynamics of DOM–POM equilibrium in seawater.

6.2. Biogeochemical cycling

The existence of significant quantities of organic matter as a gel phase has important effects on elemental cycling. We need to know more about the processes occurring within the unique chemical environment of gel interstices and on the availability and significance of gel surfaces as sites for adsorption of heavy metals and organic molecules. Moreover, gels can be consumed by some zooplankton (Passow and Alldredge, 1999; Ling and Alldredge, 2003; Orellana et al., 2003), as well as microbes, but their role in marine food webs and remineralization processes is poorly known.

6.3. Production and destruction

An exciting and important area for future research exists where the theory of gel physics and coagulation theory interface in natural aquatic systems. Clearly, a fully satisfying and accurate explanation of gel formation across the complete size spectrum from individual macromolecules to TEP aggregates will

require the incorporation of a coagulation theory. As a matter of fact, all the published models of particle dynamics in the ocean are often based on untested assumptions and portrayed solely as coagulation processes. The incorporation of the theory and methods of polymer gel physics, especially at the critical size juncture between DOM and POM, where polymers play such a significant role, will be required to develop the next generation of particle dynamics models. Both theories are required to fully understand the complex and diverse nature of particle dynamics in the ocean.

The effect of destructive processes on the abundance, chemical nature, and fate of marine gels is still poorly known but ripe for investigation. Such processes include microbial solubilization and remineralization, physical disaggregation, impacts of the internal and external chemical environment on gel properties, photooxidation (Orellana and Verdugo, 2003), and consumption by protists and zooplankton (Passow and Alldredge 1999; Orellana et al., 2000; Ling and Alldredge, 2003).

6.4. Particle dynamics and particle flux

Gels are particularly important for particle flux in that they provide an abiotic mechanism to move organic molecules up the particle size spectrum to sizes capable of sedimentation and eventual sequestration in the deep sea. A significant proportion of carbon fixed by phytoplankton can be released as assembled gel-forming polysaccharides and macrogels, which have been implicated as critical in the aggregation of diatom blooms (Passow and Alldredge, 1994; Logan et al., 1995) and the formation of marine snow (Alldredge et al., 1993). A quantification of the abundance, role, and chemical contribution of gels as glues facilitating the formation of sedimenting POM is needed, which incorporates integrated models of gel formation and particle dynamics.

6.5. Microbial microhabitats

Marine gels could have a dramatic effect on organic matter cycling via the microbial loop, yet few studies have examined these effects directly. Free-living bacteria utilizing DOM molecules and inorganic nutrients, as well as the motile protozoa grazing on those bacteria, face the challenge of intercepting their dispersed energy sources in a world of viscous (low Reynolds number) water. Under such circumstances, the efficiencies of DOM uptake by bacteria and bacterial grazing by protists often become limited by low concentrations (e.g., large average distances) and restricted mobility (Jumars et al., 1993). By creating nanometer-scale microenvironments, where macromolecules spontaneously (and continually) gather into concentrated networks, gels might profoundly alter these spatial constraints and fundamentally change the distributions and interactions of the nutrients and organisms that sustain the microbial loop (Kepkay, 1994). Patchiness at this fine scale could greatly promote, or inhibit, material and energy flows in the ocean, depending upon whether organic matter (and bacteria) associated with different types of gels are more or less readily utilized. Given the fundamental importance of the microbial loop in the marine ecosystem, marine gels might have effects that scale to higher trophic levels and global biogeochemical cycles in the oceans.

Acknowledgments

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