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Ultraviolet radiation blocks the organic carbon exchange between the dissolved phase and the gel phase in the ocean

Mónica V. Orellana
The Institute for Systems Biology, Seattle, Washington 98103

Pedro Verdugo[1]
Biocomplexity Program, Dept. of Bioengineering, University of Washington Friday Harbor Laboratories,
620 University Road, Friday Harbor, Washington 98250

Abstract

Dissolved organic carbon (DOC) is one of the major reservoirs of active organic carbon on Earth. Although the bulk of the marine DOC pool is largely composed of small refractory polymeric material, new evidence suggests that ~10% of the DOC pool (10^{16} g C) can enter the microbial loop by forming microscopic gels that can eventually be colonized and degraded by bacteria. Marine microgels result from a spontaneous and reversible assembly/dispersion equilibrium of DOC polymers forming hydrated Ca-bonded tangled polymer networks. Here we test the hypothesis that ultraviolet (UV) photocleavage should strongly inhibit the formation of microgels, because the stability of tangled networks decreases exponentially with polymer length. Because of the loss of ozone shielding, the UV-B spectral component of solar radiation ($\lambda = 280$–320 nm) has undergone a dramatic increase in the past few decades, particularly in the polar regions. We used dynamic laser-scattering spectroscopy and flow cytometry to investigate UV-induced DOC polymer cracking and the effect of UV on DOC assembly/dispersion equilibrium in 0.2 μm filtered seawater. Results indicate that exposure of seawater to UV-B fluxes equivalent to those found in Antarctica during summer solstice can cleave DOC polymers, inhibit their spontaneous assembly, and/or disperse assembled microgels. Our results agree with previous observations that indicated that fragmentation produced by UV photolysis increases exponentially with exposure time and suggested that UV could limit the supply of microbial substrate by hindering microgel formation. UV cleavage yields short-chain polymers that do not assemble and could eventually account for the old refractory DOC pool found in seawater.

The ocean holds one of the largest stocks of organic carbon on earth, playing a major role in global biogeochemical carbon cycling (Druffel et al. 1992; Hedges 1992). However, the mechanisms of transformation and degradation of this massive carbon reservoir remain poorly understood (Kepkay 1994; Amon and Benner 1996; Benner 2002). The bioreactivity of marine organic carbon varies broadly. Regardless of their chemical nature, low-molecular-weight dissolved organic carbon (DOC; <1,000 D) exhibits the lowest bioreactivity, whereas high-molecular-weight DOC and colloids support the bulk of marine heterotrophic microbial production (Kirchman et al. 1991; Kepkay 1994; Amon and Benner 1996). These features suggest that bioreactivity might be more dependent on quaternary conformation of larger molecules or on supramolecular association of smaller chains than on the presence of particular functional groups (Azam 1998; Azam and Long 2001). Recent observations lend strong support to the idea that an important fraction of the DOC pool is in dispersion/assembly equilibrium, forming supramolecular networks that might indeed play a critical role in bioreactivity (Chin et al. 1998; Wells 1998). Polymers present in the DOC fraction of seawater samples obtained from a broad range of sources can assemble spontaneously, forming the matrix of microscopic hydrogels (Fig. 1). These gels contain polysaccharide, lipid, protein, and nucleic acid chains and can range from colloidal size (100–200 nm) to 6–8 μm (Chin et al. 1998). DOC polymer assembly is reversible, exhibits the characteristic sigmoid time course of high-order kinetics, and at equilibrium has a thermodynamic yield of ~10% (Chin et al. 1998). If the studied seawaters are typical of the global DOC pool (7×10^{17} g C; Hedges 1992), then up to 7×10^{16} g C of organic carbon may occur in gel phase in the ocean. Within the scaling of the ''size-related bioreactivity'' of DOC (Kirchman et al. 1991; Amon and Benner 1996; Benner 2002), this huge mass of microgels and colloidal nanogels represents the biggest dimensional domain and the largest and most readily available pool of biodegradable organic carbon accessible to bacteria. Marine bacteria are known to readily degrade a large fraction of DOC produced by phytoplankton blooms (Kirchman et al. 1991). The advantage of gels as a microbial substrate can be explained because the ability of bacteria to cleave larger polymers into low-molecular-weight monomers—that can be rapidly incorporated and metabolized—relies on exoenzymes that are released by bacteria to the medium. The yield of the exoenzymes is higher when bacteria colonize polymer gel networks that contain a high concentration of substrate than when released to seawater that contains much lower

[1] Corresponding author (verdugo@u.washington.edu).

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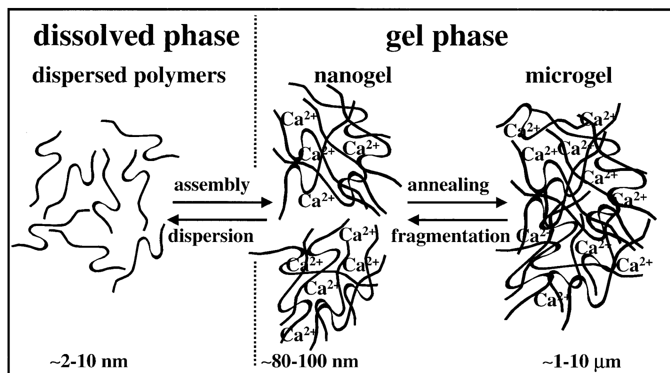

Fig. 1. Dynamics of self-assembling polyanionic marine polymer gels (from Chin et al. 1998 and Wells 1998, with modifications). Hydrogels consist of a three-dimensional polymer network imbedded in water. Water prevents the collapse of the network, and the network entraps the water. Polyanionic polymer moieties found in the dissolved organic matter pool can spontaneously and reversibly assemble, forming nanometer-sized tangled networks that are stabilized by Ca^{2+} bonds. The tangled nature of these nanogels allows polymers to interpenetrate neighboring gels, annealing into larger microgels. The size and stability of these gels depend on the charge density, hydrophobic/hydrophilic properties, length of the assembled polymers, and the kinematics of shear forces prevailing in seawater. The chemical and physical features of the individual polymer chains and the dielectric properties of the entrapped seawater determine the topological dynamics, the chemical and physical reactivity of the network, and how they interact with living organisms. Close intermolecular distances and hindered mobility produce a unique set of emergent properties that are different from those of the dispersed polymers that make up these networks. Polymer chains inside microgels are in a statistically stable neighborhood, creating microenvironments of high-substrate concentration that remain in thermodynamic equilibrium with the surrounding medium and serve as a rich source of substrate to microorganisms.

concentrations of substrate (Azam 1998; Azam and Long 2001). Taken together, these ideas suggest that the marine gel phase derived from the assembly of DOC polymeric material must play a critical role in biogeochemical cycling (Wells 1998). Thus, events that interfere with DOC polymer assembly could potentially arrest the formation of microgels, blocking a critical route of microbial processing in the ocean. Here we show that high-energy ultraviolet (UV)–B light can readily cleave DOC polymers and effectively arrest the formation of microgels.

Methods

Sample collection and DOC measurement—Seawater from the Puget Sound (Washington) and Newport Bay (Oregon) was gravity filtered through a sterile tandem of GF/F fiberglass membrane and 0.22 μm Millipore (MillexR-GV; PVDF Durapore) filter (prewashed with 0.1 N HCL) and stored in clean sterile bottles in the dark at 4°C for 1–4 weeks. Samples were acidified, and DOC was measured in a Shimadzu TOC-5000 analyzer using potassium hydrogen phthalate as the standard. The absence of bacteria in the filtered seawater was confirmed by dynamic laser-scattering spectroscopy (DLS) using a Brookhaven Spectrometer (Brookhaven Instruments) and by flow cytometry using an Epics Profile cytometer. Flow cytometry directly counts fluorescently labeled bacteria by detecting their emission as the cells are entrained in a laminar flow and led in a rapid sequence through a narrowly collimated laser beam. In this case, we fluorescently labeled bacterial nucleic acid using a 5 μmol L^{-1} concentration of Cyto Green ($\lambda_{emission}$ = 530 nm; Molecular Probes). In addition, the forward angle light-scattering mode of the flow cytometer was used to detect particle size. Calibrations conducted at increasing dilution in suspensions of 500 nm fluorescent microspheres indicated that, at this size range, the sensitivity of flow cytometry to detect particles was virtually infinite, reaching up 1 particle ml^{-1}, but that detection takes a proportionally longer time with decreasing bacterial concentrations.

DLS detects motion, in this case relying on the difference between the motion of molecules or particles like gels—which exhibit classical Gaussian random walk diffusional mobilities with a Loretzian spectral distribution—and bacteria that move in a characteristic Markovian stop-and-go pattern. Unlike flow cytometry, DLS does not provide bacterial counting, because it relays in the probability of bacteria crossing a small scattering volume. However, it is an extremely sensitive method that can reach up to nanomolar detection ranges and extend down to nanometer size scale (Cummins 1972).

UV-B and UV-A calibrations—The spectral distribution of a Spectroline UV-B aged lamp (model XX-15B; Spectronics) and an aged UV-A lamp (model XX-15A; Spectronics) were verified with a spectrofluorometer (Shimadzu RF 500U). The intensity of the lamps was measured with a 0.2-nm resolution spectroradiometer (LiCor LI-1800W, cosine sensor) calibrated with a stable LI 1800-02 lamp. The measurements were done in air and corrected every 2 nm by the immersion factor given by the manufacturer. In the experiments, the UV-B lamp was aimed at a 90° angle directly on top of 4 cm high × 24 mm diameter cylindrical scattering cells that contained the seawater samples. The cells were covered with a 3 mm cellulose acetate filter to block the shorter wavelengths not found in nature. Similarly, UV measurements were conducted by placing the photo detector—covered with the acetate filter—outside the scattering cells, aimed at the UV source, and at a distance from the source identical to the distance that the air-water interface of the seawater was located in the irradiated samples.

Microgels and DOC polymer sizing—Detailed protocols to measure the hydrodynamic diameter of free DOC polymers and to monitor the assembly of marine microgels by DLS have been published elsewhere (Chin et al. 1998). In brief, the quartz scattering cells containing seawater samples were positioned in the goniometer of a Brookhaven Ar-ion (λ = 488 nm) laser spectrometer (Brookhaven Instruments). The autocorrelation function of the intensity fluctuations of the scattered light collected at a 45° angle was processed by a Brookhaven BI 9000 AT autocorrelator and averaged over a 10-min sampling time. Particle size distribution was calculated using the CONTIN method (Provencher 1982). Experimental and control measurements were done in triplicate

in 10-ml seawater samples at room temperature. Additional measurements of microgel size and concentration were performed in the same seawater samples, using a Coulter Epics Profile flow cytometer. In this case, the assembled gels were labeled with Nile red (Molecular Probes). Calibrations of both methods were conducted using standard monodisperse suspensions of latex microspheres (Polysciences) ranging from 50 nm to 6 μm for DLS and 500 nm to 10 μm for flow cytometry. Although flow cytometry can measure both the size and concentration of particles, the low sensitivity of the instrument (Epics Profile) in the low-nanometer range of particle size precluded measurement of the concentration of gels smaller than 600 nm.

UV-A and UV-B exposure of free DOC polymers—Seawater samples were shaken and refiltered through a 0.22 μm Millipore filter (MillexR-GV; PVDF Durapore) prewashed with 0.1 N HCL. As in previous experiments (Chin et al. 1998), DOC polymer assembly and microgel formation were prevented by chelating the seawater Ca^{2+} with 10 mmol L^{-1} ethylene diaminetetraacetic acid (EDTA) or by dialyzing the seawater samples against artificial seawater without Ca^{2+} using a 100-D molecular-weight cutoff membrane. Samples were then exposed for 24 h to UV-A (10 W m^{-2}, λ = 320–395 nm) and for 0.5, 1, 3, 6, and 12 h to UV-B radiation (0.5 W m^{-2}, λ = 280–320 nm). UV exposure was performed in triplicate, at 20°C in air atmosphere using 10-ml quartz SUPRASIL 300 UV-transparent scattering cells with a light transmittance between λ = 150 and 5,000 nm (HELLMA). Seawater samples maintained in the dark served as controls.

DOC polymer assembly—Assembly of free DOC polymers in both irradiated and non-irradiated seawater aliquots was initiated by restoring the [Ca^{2+}] to normal levels found in seawater (10 mmol L^{-1}). The hydrodynamic diameter of the assembled microgels was monitored by DLS every hour for the next 10 h and verified every 24 h during the next 15 d. To rule out potential artifacts produced by the effect of degradation products derived from the UV irradiation of EDTA, we verified that the kinetics of assembly of the samples in which Ca^{2+} was chelated with EDTA was similar to samples from which Ca^{2+} was removed by dialyzing the seawater samples against Ca^{2+}-free artificial seawater (data not shown). In both cases, DOC polymer assembly was initiated by restoring the Ca^{2+} concentration to 10 mmol L^{-1}.

Microgel exposure to UV radiation—The microgels resulting from both 60 min and 6 h irradiated DOC and from nonirradiated DOC samples were then reexposed for 24 h to UV-A (10 W m^{-2}, λ = 320–395 nm) and for 0.5, 1, 3, 6, 12, 18, and 24 h to UV-B radiation (0.5 W m^{-2}, λ = 280–320 nm). After each irradiation period, the hydrodynamic diameter of the gels was monitored by DLS.

Results

The effects of UV radiation on freely dispersed (nonassembled) DOC polymers and on assembled microgels were investigated by DLS and flow cytometry (FC). Polymers in the DOC pool can spontaneously assemble into Ca^{2+}-bonded

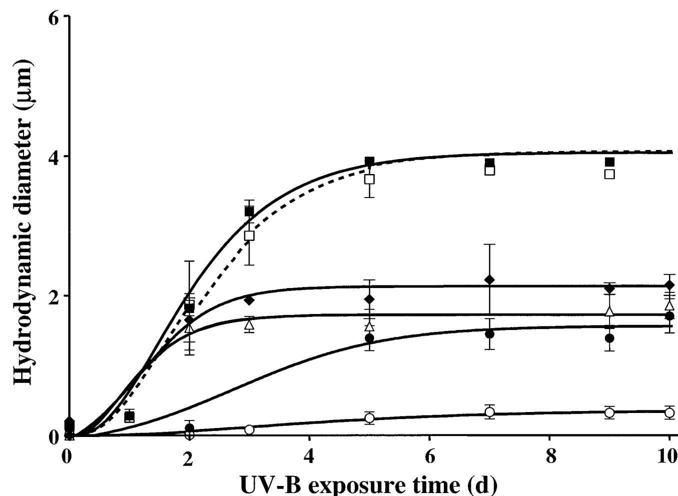

Fig. 2. Spontaneous assembly of DOC assembly follows a characteristic sigmoidal second-order kinetics, forming polymer gels that grow from colloidal nanometer size to multimicron size in ~60 h. Data points in filled squares represent the assembly of nonirradiated DOC polymers. Assembly of DOC polymers irradiated for 24 h with UV-A (10 W m^{-2}, λ = 320–400 nm) are in open squares. Note that there is no statistical difference between the assembly kinetics of controls and UV-A–irradiated samples. The assembly of DOC polymers in seawater samples exposed to UV-B (0.5 W m^{-2}) for 30 min (filled diamonds), 1 h (open triangles), 6 h (filled circles), and 12 h (open circles) follow a similar second-order kinetic profile, but the time to equilibrium is longer and the equilibrium size is smaller. Data points correspond to the mean ± SD of 30 DLS measurements.

polymer networks that form the matrixes of microscopic gels (Chin et al. 1998). To study the effect of UV in dispersed (nonassembled) DOC polymers, we prevented assembly by dialyzing samples of 0.22 μm–filtered seawater against Ca^{2+}-free artificial seawater. As has been shown elsewhere, DOC polymers form a polydisperse collection with hydrodynamic diameter reaching up to 2.5 nm (Aluwihare et al. 1997; Chin et al. 1998). The average size of DOC remained unchanged after a 6-h exposure to 10 W m^{-2} UV-A (λ = 320–395 nm). The exposure of seawater to UV-B (λ = 280–320 nm) in irradiation regimes similar to the average found at the summer solstice in Antarctica (0.5 W m^{-2}) (Holm-Hansen et al. 1993) decreased the size of DOC polymers to dimensions below the resolution of our laser spectrometer (<1 nm). These results agree with previous observations that indicated that fragmentation produced by UV photolysis increases exponentially with exposure time (Allard et al 1994). The cracking of DOC chains can take place by direct photochemical reaction (Mopper et al. 1991; Moran and Zepp 1997) and/or random cleavage by UV-generated free radicals (Blough 1997; Mopper and Kieber 2001).

The effect of UV on the ability of DOC polymers to self-assemble was investigated in controls kept in the dark and in water samples exposed to UV for periods of time ranging from 30 min to 12 h (Fig. 2). In both UV-irradiated and nonirradiated samples, the assembly was initiated by increasing the concentration of Ca^{2+} to 10 mmol L^{-1}, as is normally found in seawater. The assembly kinetics and the equilibrium

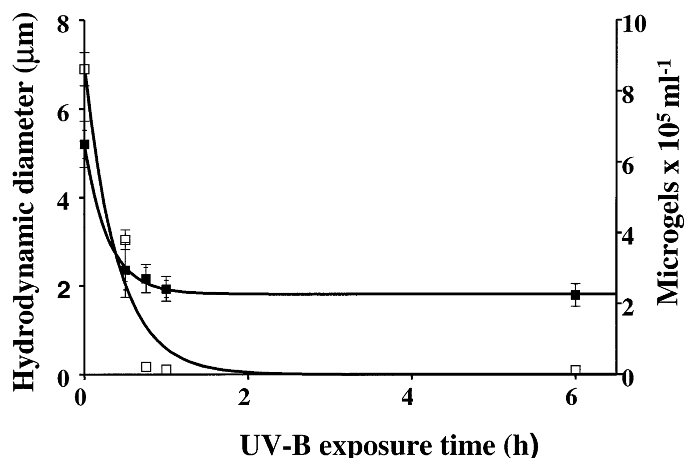

Fig. 3. Depending on the time of UV-B exposure received by the DOC polymers before assembly, the equilibrium size of the resulting microgels (filled squares) and their concentration (open squares) decreased exponentially. Note that the concentration of nanogels resulting from the assembly of DOC polymers irradiated for 12 h are not reported because the low resolution of flow cytometry in the nanometer size did not allow a reliable count. Data points correspond to the mean ± SD of 30 DLS and flow cytometry measurements.

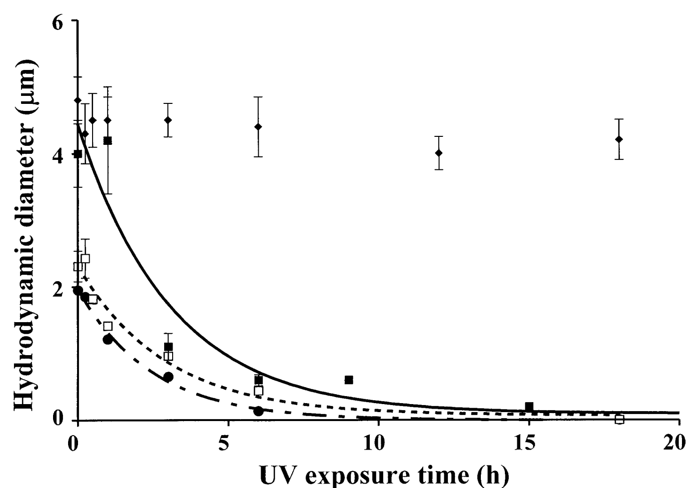

Fig. 4. Although the hydrodynamic diameter of microgels exposed to UV-A remain fairly constant (filled diamonds), UV-B exposure of assembled microgels resulted in a single exponential decrease of their size. Microgels assembled from nonirradiated DOC polymers (filled squares) or from DOC polymers preirradiated for 30 min (open squares) and 6 h (filled circles) all virtually dispersed in 5–10 h of UV-B exposure. However, the characteristic time of dispersion decreased with increased exposure to UV-B radiation.

concentration of assembled microgels resulting from DOC samples exposed to UV-A were not significantly different from controls and from values reported elsewhere in nonirradiated seawater (Chin et al. 1998). Both exhibit a characteristic sigmoid time course that yields microgels of ~4–6 μm in ~60 h (Fig. 2). However, DOC assembly in samples exposed to UV-B yielded fewer and smaller microgels and took a longer time to reach assembly equilibrium. The equilibrium size and concentration of microgels decreased exponentially with increasing time of the UV-B exposure of DOC polymers (Fig. 3). This outcome can be readily explained by polymer theory as the stability of tangled networks—like those found in DOC-assembled microgels—depends on the second power of polymer length (Edwards 1974; de Gennes and Leger 1982). These results are also consistent with previous observations that indicated that UV-B–induced DOC fragmentation increases exponentially with exposure time (Mopper et al. 1991; Moran and Zepp 1997). Depending on the extent of cleavage, UV-B–irradiated DOC polymers assemble into smaller, less stable submicron-size gels that fail to anneal to each other. Failure to anneal can explain the decrease of hydrodynamic diameter and microgel concentration with increased exposure to UV-B (Fig. 3). At the limit, shorter chains resulting from DOC irradiated for >12 h yield only nanometer-sized colloidal gels (Fig. 2, open circles). In theory, in UV-B–irradiated seawater colloidal-sized nanogels should reach higher concentrations than in nonirradiated seawater. However, although we could reliably verify particle size by DLS, the low resolution of our Epics Profile flow cytometer for particle sizes <600 nm prevented us from conducting reliable particle counts to verify the nanogel concentration in these samples.

UV can also lead to a loss of polyanionic charge (Francko 1986), resulting in decreased Ca^{2+} bonding and a corresponding decline in polymer network stability. However, although there is reliable physical theory to predict that UV-B–induced cleavage can inhibit microgel formation (Edwards 1974; de Genes and Leger 1982)—and our data show that UV-B can cleave the polymeric material found in DOC—the extent to which a potential photochemical loss of polyanionic charge could additionally contribute to the destabilization of assembled DOC cannot be ascertained from our observations.

While the outcome of UV-cleaved free polymer chains is failure to assemble (Edwards 1974; de Genes and Leger 1982), the potential outcome of exposure of preassembled networks to UV is twofold: it could either break the polymers and disperse the gels or, conversely, crosslink the network, making the gels more stable (Maeda et al. 1994). To investigate the effect of UV on already assembled gels, the same microgels assembled from nonirradiated DOC polymers and from 60-min and 6-h irradiated DOC shown in Fig. 2 were reexposed to UV-A (10 W m^{-2}) for 24 h and to UV-B (0.5 W m^{-2}) for 0.5, 1, 3, 6, 12, 18, and 24 h (*see* ''Methods'' section).

The hydrodynamic diameter and concentration of nonirradiated and UV-A–exposed microgels remained fairly constant, whereas the hydrodynamic diameter of UV-B–irradiated gels and their concentration decreased exponentially with an increasing time of UV-B irradiation, reaching full dispersion after 12 h (Fig. 4). The characteristic single exponential decrease of microgel size and concentration suggests that microgel dispersion probably results from a diffusion-driven process whereby UV-B–cleaved DOC polymers move directly out of assembled networks rather than from breaking of microgels into intermediate smaller particles.

Discussion

Although the flux of UV-A over the Earth has remained stable in the past few decades (McKenzie et al. 1999), the flux of solar UV-B radiation is rising, probably because of the depletion of the atmospheric ozone layer (Mackenzie et al. 1999). Short-wavelength UV-B radiation (λ = 280–320 nm) has been estimated to increase as much as 14-fold over the Antarctic during the austral spring (Mackenzie et al. 1999). Similar trends have been observed in the Arctic and in temperate and tropical oceans (Stolarski et al. 1992). The present results show that UV-B exposure at fluxes that are equivalent to or smaller than those measured at the ocean surface (Frederick et al. 1989) can drastically disrupt the exchange between the dissolved and the gel phases in seawater. Cleavage of DOC by UV-B can avert polymer assembly and the formation of microgels or induce the dispersion of preassembled microgels.

The size distribution of marine organic matter is highly skewed toward smaller components (Benner, 2002), with ''truly dissolved'' substances making up ~75% of total organic carbon and the high-molecular-weight fraction essentially making up the remaining 25%. Polymer theory can readily explain the observation that higher-molecular-weight DOC and colloids have faster rate of biodegradation and turnover than low-molecular-weight DOC (Amon and Benner 1996), because the probability of assembly of polymer gels and the stability of the assembled gels decreases with the second power of the polymer length (Edwards 1974; de Gennes and Léger 1982). Low-molecular-weight polymers too large to move across the bacterial membrane but too small to assemble into microgels should remain dispersed in solution, probably joining the DOC refractory pool. Conversely, high-molecular-weight DOC can assemble, forming microgels that provide an optimum microenvironment of substrate for bacteria to colonize, release their exoenzymes, and harvest small molecules at high local concentrations inside the gel's matrix (Azam 1998; Azam and Long 2001). This idea is consistent with the fact that photochemicaly degraded humic substances, which assemble into microgels (Benedetti et al. 1996), stimulate bacterial growth (Bertilsson and Allard 1995; Jorgensen et al. 1998), whereas short and small molecules that do not assemble into forming microgels (glucose) do not increase bacterial abundance (Kirchman et al. 1990). The counter argument to these observations is that UV radiation can also increase bacterial production by releasing pyruvate and other low-molecular-weight bacterial substrates (Kieber et al. 1989). The explanation for this apparent paradox is that UV photolysis produces a random fragmentation of high-molecular-weight DOC polymers, yielding low-molecular-weight products that, in a short timescale, can be readily incorporated and metabolized by bacteria (Mopper at al. 1991; Moran and Zepp 1997; Mopper and Kieber 2001). However, UV cleavage of polymers also yields DOC chains too large to permeate the bacterial membrane yet too short to assemble into stable networks that, in a longer timescale, bacteria can colonize and efficiently degrade with their exoenzymes.

The thermodynamic yield of DOC assembly suggests that marine microgels are likely to form a major pool of bioactive material in the ocean. At equilibrium, ~10% of DOC assembles into microgels (Chin et al. 1998). In a global DOC pool of $\sim 7 \times 10^{17}$ g C (Hedges 1992), microgels would account for $\sim 7 \times 10^{16}$ g of the organic carbon found in the ocean. This gel phase mass, which does not include a much larger pool of coexisting nanogels, exceeds the total biomass of marine organisms by a factor of 50 (Hedges and Oades 1997). Given that an average polymer gel is 1%–2% weight solid material, the total volume of microgel in the ocean should reach $\sim 7 \times 10^{15}$ liters.

Although the rates at which bioactive elements pass through the marine microgel (and nanogel) pools are unknown, the similar day-to-week timescales for microgel formation (Chin et al. 1998) and ^{234}Th pumping from colloidal to particulate size (Moran and Buesseler 1992; Guo and Santschi 1997) suggest that the corresponding fluxes could be huge. Although the penetration of UV in seawater is limited, the present observations strongly suggest that increased UV-B radiation could be affecting an unsuspected submicron level of ''patchiness'' (Azam 1998) in the ocean gel phase that could influence the passage of DOC to microorganisms by the marine microbial loop (Azam et al. 1983). Perturbations in the dynamics and budgets of DOC polymer assembly could influence a broad range of oceanographic processes, ranging from the accumulation of short-chain refractory old DOC polymers that remain dispersed (unassembled) (Bauer et al. 1992; Aluwihare et al. 1997) or assembled in 100–200 nm-sized colloids, which are too small for bacteria to colonize (Koike et al. 1990; Wells and Golberg 1991, 1992), to changes in trophic dynamics and carbon cycling (Wells 1998), or to climate changes by alteration of atmospheric carbon concentrations (Siegenthaler and Sarmiento 1993).

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