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Dynamics of marine biopolymer networks

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Summary

Regardless of their size, composition or origin, assembled biopolymers form networks that exhibit a unique set of emerging properties that account for phenomena ranging from biopolymer networks in secretory granules and their role in storage and release of hormones [1], to self-assembly of marine biopolymer and their role in carbon cycling in the ocean [2]. Our goal is to use the power of established polymer physics theory to develop new models and better predictive tools in these different fields. In this paper I'll briefly review some highlights of our work on biopolymer self-assembly in the ocean and the significance of the marine gel phase on global carbon cycling.

Introduction

The coupled biological, chemical, and physical processes by which bioactive elements are cycled in the ocean represent one of the most intricate and critical systems on Earth. The understanding of how carbon is cycled in our planet is critical to the future of humans and other species. At the present time we have an estimated annual overload of ~ 3.5 gigatons (1 Gt = 10^{15} g) of CO_2 discharged to our atmosphere. Policies to effectively limit fossil fuel burning have so far proved to have limited reception among industrialized countries. In addition, the processes whereby carbon is cycled remain poorly explored. Atmospheric CO_2 is removed mainly by photosynthesis. About 70% of the surface of our planet is covered by seawater and half of the global recapture of CO_2 takes place in the seas. The output of the remarkably efficient photosynthetic machinery of phytoplankton – one of the major photosynthetic species in our planet – is released to the seawater contributing to the formation of what the geochemist call Dissolved Organic Carbon pool (DOC). DOC is among the largest masses of reduced organic carbon present in our planet, reaching ~700 gigatons. This is equivalent to the mass of carbon in atmospheric CO_2 and only slightly smaller than the amount of carbon found in terrestrial biomass and soil humus [3]. However, the fate of these molecules, their chemical, physical, and biological

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interactions and their ultimate destination remain as one of the most exciting and significant challenges in geochemistry and marine biology. Although geochemical studies reveal that DOC is made of a heterogeneous mix of biopolymers – largely polysaccharides – theory and methods of polymer physics had not been used to study the polymer dynamics of seawater DOM. The first application of the remarkable predictive power of these tools lead to the striking discovery that DOM polymers can spontaneously self-assemble forming the matrix of microscopic gels (Fig 1) [2].

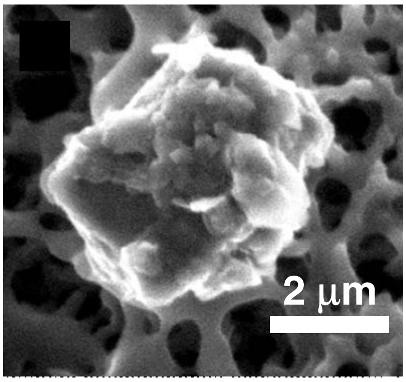


Figure 1. Environmental Electron Microscopy allows to visualize with nanometer resolution fully hydrated unfixed preparations, including living cells. The micrograph shows a self-assembled marine microgel on the surface of a porous filter seen in the background. Notice that the gel is made out of annealing of smaller gels of submicron dimensions.

These findings have fundamentally changed how oceanographers think about processes linking the microbial and biological phenomena taking place in the ocean to the biogeochemical dynamics of the rest of the biosphere and the geosphere [4]. The characteristic features of these self assembled microgels (SAM) forming porous networks, their unique ion exchange and phase transition properties, and their ability to provide a rich substrate and protected environment for marine bacteria makes these gels one the most intriguing and exciting subjects in oceanography.

SAM are physical hydrogels containing a tangled polymer matrix stabilized by Ca bonds. As shown in Fig 2, both dynamic laser scattering and flow cytometry indicate that DOC self-assembly follows a characteristic second order kinetics. DOC assembly is inhibited by chelation of Ca, it is reversible, and exhibit a thermodynamic yield at equilibrium of ~10 % [2].

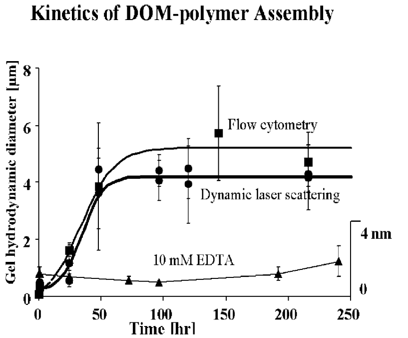
A two step assembly kinetics of the form:

$$\text{DOC } (D_C) \quad \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} \quad \text{Nanogel } (N_C) \quad \underset{k_{-2}}{\overset{k_2}{\Leftrightarrow}} \quad \text{Microgel } (M_C)$$

where

$$-\mathrm{d}[D_C]/\mathrm{d}t = k_1[D_C] - k_{-1}\,[N_C]$$
$$\mathrm{d}[N_C]/\mathrm{d}t = \mathrm{k}_1[\mathrm{D}_C] - k_{-1}\,[N_C] - k_2\,[D_C]^2 + k_{-2}\,[M_C]$$
$$\mathrm{d}\,[M_C]/\mathrm{d}t = \mathrm{k}_2\,[D_C]^2 - k_{-2}\,[M_C]$$

predicts that in the first 20-30 min self-assembly must result in the formation of nanogels (Fig 3) that then anneal to form aggregated microgels as those illustrated in Fig 1.



Chin W-C, M., Orellana, & P. Verdugo (1998) *Nature* 391,586

Figure 2. The time course of marine polymer assembly leading to the formation of microscopic gels follows characteristic second order kinetics. In this case seawater was first filtered by a 0.2 µm filter. The spontaneous assembly of the free DOC polymers present in the filtrate results in a time dependent particle growth that was monitored by both Dynamic Laser Scattering and Flow Cytometry. Notice that chelation of Ca by EDTA results in complete inhibition of the self-assembly process.

Although Ca is mostly bound, it reaches much higher concentrations inside SAM than in seawater. Polarized microscopy revealed that small changes of seawater pH or temperature can induce volume phase transitions, and the formation of crystalline complexes (Fig 4) rich in crystalline/organic complex material that can drastically increase SAM's sedimentation coefficient. These processes can contribute to the formation of organic-enriched sediments and eventually to the sequestration of carbon in the deep sea and its corresponding withdrawal from the global carbon cycle.

Polymer gel theory indicates that the size of self-assembled gels and the thermodynamic yield of mass transfer should increase with the second power of the length of the polymers that make their matrix [5, 6]. The experimental test of these principles shows that short polymers resulting from bacterial biodegradation or ultraviolet photo-cleavage of DOM do indeed fail to self-assemble [7, 8]. Figure 5 illustrates the progressive decrease in assembly kinetics - monitored by dynamic laser scattering - of DOC polymers exposed for 0, 30 min, 1, 2, 3, 6, and 12 h to 0.5 W of UV radiation (λ = 280-320 nm).

These observations are significant because the flux of short-wave solar UV-B radiation is increasing, likely due to the depletion of the atmospheric ozone [9]. The Antarctic has been reported to suffer during the austral spring a ~14-fold increase of UV-B radiation (λ = 280–320 nm) [9]. Equivalent observations have been reported in the Arctic and in and tropical oceans [10].

Our studies indicate that equivalent to or smaller UV-B fluxes than those measured at the ocean surface [11] can radically disrupt the kinetics and thermodynamic yield of the assembly equilibrium of DOC eventually turning off microgel formation in sea water.

The thermodynamic yield and rates of DOM assembly↔dispersion equilibrium measured in the laboratory [2] are consistent with measurements of ^{234}Th pumping from colloidal to particulate size [12, 13] suggesting that the corresponding fluxes could be enormous. At a 10% DOM-SAM transfer rate from a 7×10^{17} g C DOC pool means that the mass of SAM in the ocean must reach 7×10^{16} g C. This mass is ten to fifty times larger than the global biomass of marine organisms [14] and is among the richest pools of bioreactive carbon accessible to microbial degradation [15]. In fact, one of the most relevant features of these gels is that they represent the entry point of marine carbon cycling. Because gels form porous niches containing high concentrations of potential substrate, they support a microbial ecosystem that is fundamentally different in function and dynamics from the conventional marine model. DOC biopolymers are degraded by bacteria that directly or indirectly make the

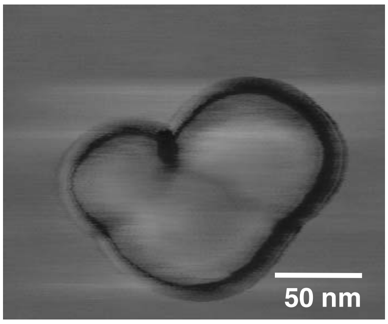


Figure 3. Samples of seawater obtained during the first few minutes of the self-assembly process illustrated in Figure 2 contain nanogel particles as revealed by atomic force microscopy. Because of the tangled topology of these networks the polymers that make them can undergo reptational diffusion interpenetrating neighboring gels leading to annealing and formation of larger gels. In this illustration, three nanogels are annealed together.

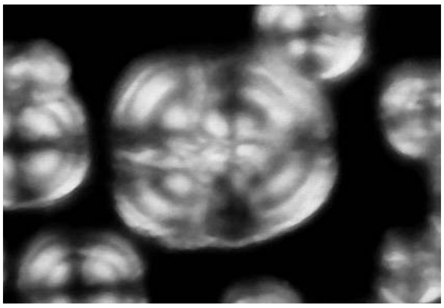


Figure 4. The strong polyanionic properties of DOC self assembled networks results in Donnan partition and accumulation of Ca inside marine microgels. Small changes in temperature or pH that don't affect Ca solubility in seawater can produce formation of $CaCO_3$ crystals inside the gels as shown by polarized microscopy in this illustration.

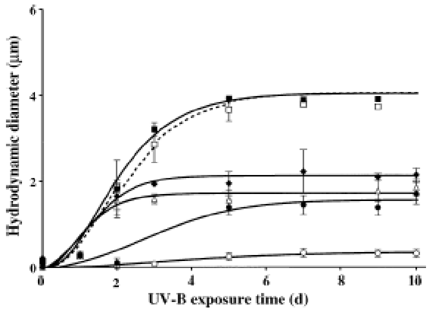


Figure 5. Exposure of seawater to UV-B at fluxes similar to those found in the Antarctic oceans during summer results in progressive inhibition of DOM self-assembly as shown in this illustration. (White and black squares □ are controls, black ◊, Δ, •, and ° correspond to UV-B exposure for 0.5, 0.75, 1, 6, hr. respectively).

basic food stock of other higher species. However, DOC polymers need to be cleaved substrates to < 600 MW before they can be imported into the bacteria. Because the average concentration of DOC is only ~25 μM and bacterial degradation relies on ectoenzymes, the rate limiting parameter for bacterial digestion is the diffusion of substrates and enzymes in seawater. In theory bacterial cleavage of DOM should be highly inefficient. However, SAM are porous structures that can be readily colonized and cleaved by bacteria offering a simple explanation for the quick bacterial turnover of DOM. A critical implication of this outcome is that since the metabolic machinery of marine bacteria can re-oxidize carbon the ocean can no longer be regarded–as previously believed–to be a buffer for the global overload of CO_2 taking place in our planet.

In summary, SAM's consist of interwoven organic polymers stabilized by physical linkages forming three-dimensional porous networks that confine solvent molecules and other solutes. They enclose nanoscale microenvironments that exhibit radically different emerging physical, chemical, and biological properties from those of the dispersed component molecules resulting in significantly different chemical reactivities and biological availabilities. Small changes in ionic concentration, ambient pH, or temperature can trigger quick phase transitions in gels that can drastically change their densities, permeabilities, dielectric properties, and chemical reactivities; and thus their potential interactions with enzymes and living organisms. The unique features of these networks to continuously assemble and disperse, binding (and releasing) a broad range of molecules and ions into a matrix of concurrently varying volume, porosity, and structure ranks gels among the most heterocomplex systems in nature. The ramifications of microgel formation in the ocean might well scale nonlinearly through the microbial system to the world ocean and global climate system [4]. If in fact, even a few percent of marine DOM components are present as gels in seawater, then oceanographers will have to thoroughly revise the mechanistic basis of such diverse aquatic paradigms as the "microbial loop", colloid pumping, trace metal scavenging, humification and size-reactivity relationships. In a gel-containing ocean holding a heterogeneous and dynamic substrate distribution at the micron scale, the suitability of assigning static bulk properties such as concentration, molecular size and age of the moieties found in seawater could eventually comes into question [16].

In closing, the ocean represents one of the most exciting and significant challenges to polymer gel scientists, the bounty is huge, and the need to form and attract new talent into this new frontier is most critical.

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