

Characterizing the Role of Metal-Binding Moieties in the Photochemistry of Chromophoric DOM in Marine Systems

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LONG-TERM GOALS

Our long-term objective is to understand the chemical structure and cycling of metal-organic complexes in both surface and deep marine waters. In particular, we are interested in the effect of metal complexation on the optical properties and photochemical reactivity of dissolved organic matter (DOM).

OBJECTIVES

Chromophoric DOM (CDOM) strongly alters the chemical and optical properties of seawater (e.g. DeGrandpre et al., 1996; Vodacek et al., 1997), yet little is known about the identity and cycling of the compounds that impart this characteristic to marine waters. We believe that providing a chemical context for interpreting CDOM optical data is crucial. Using samples from coastal San Diego and the Southern California Bight, representing coastal and open ocean systems which are primarily influenced by marine processes (as opposed to terrestrial inputs), we plan to address the following major objectives: isolate and characterize the bulk chemical properties of low (<1 kDa; LMW) and high (>1 kDa; HMW) molecular weight fractions of CDOM; further purify and chemically characterize the LMW and HMW CDOM fractions to identify particular metal binding moieties; perform photochemical studies on metallated and de-metallated fractions and isolate components to determine the influence of metal binding moieties on the photoreactivity and ultimately, bioreactivity of CDOM.

APPROACH

Our studies so far have focused on (i) isolating total, HMW and LMW CDOM from cultures and field samples for chemical and optical characterization, (ii) identification of Fe-ligands and (iii) photochemical experiments. Currently, we have field samples from SIO pier, the North Central Pacific

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ocean, the California Current and the Eastern Equatorial Atlantic ocean. We also hope to collect more samples from the California Current and San Diego Bay in the next 6 months.

Our initial studies focused on examining variations in bulk and compound level chemical characteristics of CDOM fractions. Using solid phase extraction (SPE; polydivinylbenzene resin) alone or in combination with ultrafiltration we isolate CDOM from seawater for chemical characterization. When ultrafiltration is followed by SPE we can isolate >1kDa (HMW; not chemically biased) and <1kDa (LMW; semi-polar and non polar) fractions of CDOM and when SPE is used alone we successfully isolate semi and non-polar organic compounds from seawater. As far as we know, in marine systems, this analytical approach combining ultrafiltration with SPE extraction is unique to this study and allows us to isolate a larger portion of marine DOM than previously achieved. A similar method, using a different SPE resin was recently applied in a freshwater system (Kaiser et al., 2003). Bulk characteristics of these various fractions are determined via NMR spectroscopy, UV-Vis spectrophotometry, elemental analysis, and isotope ratio mass spectrometry (IR/MS). The LMW CDOM is further fractionated on the SPE column based on polarity (5 fractions ranging from semi- to non-polar) and subjected to the same suite of bulk analyses as above. We are attempting further separations using HPLC with photo diode array (PDA) detection to isolate individual, optically active compounds from the three most polar CDOM fractions (A, B and C). These initial HPLC separations are based on both size (size exclusion) and polarity (reverse phase). Our final goal is to use mass spectrometry, following HPLC separation, to identify optically active organic compounds bound to Fe in these fractions of CDOM. To enhance our success at targeting Fe-bound organic compounds we are also developing a method based on Fe-affinity chromatography.

Photochemical experiments are being carried out using both the field and phytoplankton culture samples to determine the photoreactivity of recently produced, and accumulating (in both surface and deep waters) CDOM. Both natural samples and samples amended with Fe are irradiated in quartz flasks under natural sunlight to examine the effect of Fe on the photoreactivity of CDOM. Changes in CDOM characteristics are monitored by dissolved organic carbon (DOC) concentration measurements, UV-Vis spectrophotometry, size exclusion chromatography and LC-MS.

The isolation and structural characterization of CDOM is being carried out by one graduate student and a post doctoral fellow (the latter is being supported on this ONR grant) under the supervision of LIA, and the Fe-ligand characterization and photochemical studies are being carried out by the post-doc under the supervision of KB.

WORK COMPLETED

As outlined above, we have collected several surface and deep water samples and are currently focusing on a sample set from the California Current (from surface and 1000 m), samples from SIO pier (to examine temporal variations) and CDOM isolated from cultures of both *T. weissflogii* and *T. pseudonana*. Samples from the California Current were isolated by passing 200L of seawater (per sample) through SPE resin and eluting in acetone and methanol. SIO pier samples and culture samples were processed as above or first ultrafiltered to isolate the >1kDa material and then passed through the SPE resin. The California Current samples represent offshore and vertical gradients in CDOM and were collected during CalCOFI's spring sampling cruise (Point Conception, CA down to San Diego, CA). Samples were taken along an offshore transect from approximately 200-500 m water depth to 2000m water depth. Pier samples were collected during different times of the year and most recently during an unusual flagellate bloom that produced large quantities of DOM, and during a non-toxic red tide (*Lingulodinium polyedrum*).

Using samples isolated from the SIO pier we have begun developing methods to isolate CDOM-Fe complexes by absorption onto an Fe-loaded IMAC resin. We have also performed some photochemistry experiments using CDOM isolated from both surface and deep (1000 m) waters to determine both intrinsic photoreactivity and the role that Fe may play in enhancing/depressing CDOM photoreactivity

RESULTS

Isolation and characterization of LMW and HMW CDOM: We have performed crude chemical separations and bulk chemical characterization of the California Current samples and some of the SIO pier samples. We have also further purified some of these samples via HPLC.

1. We have successfully shown that both ultrafiltration and SPE can isolate CDOM from the surface and deep ocean and from phytoplankton cultures.
2. UV-Vis spectra of isolates are similar to that of total DOC and are characterized by an exponential decrease in the absorbance from a maximum at low wavelengths. However, the spectrum of HMW and LMW CDOM are significantly different from each other with LMW DOM often showing shoulders at approximately 290 nm and 350 nm. Our initial deep samples generally have a higher UV absorbance and the LMW, least polar fractions have interesting shoulders at 230, 240, and 300 nm.
3. Based on the suite of samples isolated from the California Current we find that SPE fractions from the surface and deep ocean are chemically distinct, yet, there is little horizontal variation in chemical structure. In general, the deep ocean samples appear to be more reduced and rich in methylene residues.
4. Further separation (A through E; E being the least polar) of the SPE isolates as discussed above allowed us to distinguish distinct chemical signatures within CDOM (Figure 1). Across samples, individual fractions, e.g. 1A and 2A, with the same polarity have remarkably similar NMR spectra. Chemical differences between fractions A through E, as observed by NMR spectroscopy, are dominated by a decrease in α -hydroxyl (e.g. carbohydrates) and ester (e.g. acetyl) functionalities as polarity decreases. These are the first data of their kind for marine samples and significantly expand our current knowledge regarding the chemical structure of DOM. As expected, the nitrogen content of each fraction decreases dramatically with decreasing polarity and carbon content can be as high as 60% of the total mass (fraction E). Interestingly, the carbon isotopic signature ($\delta^{13}\text{C}$) of each fraction increases with polarity such that the lipid rich fractions are 2-3 per mil lighter than their most polar counterpart. This is consistent with the fractionation of carbon isotopes associated with the biosynthesis of lipids (e.g. DeNiro and Epstein, 1977). The UV-Vis spectra indicate the highest absorbance in the semi-polar fraction with little to no absorption in fraction E (the least polar fraction) except in the deep samples. All samples are a light yellow color.
5. We are currently attempting to isolate individual optically active compounds from the three most polar fractions (A through C) using both size exclusion and reverse phase HPLC. So far, we have isolated only a limited number of individual compounds that absorb at wavelengths >250 nm and we think further offline purification of each fraction is needed before separation by HPLC.
6. Reverse phase HPLC separation was more successful at isolating individual compounds (absorbing at 254 nm) from culture samples. This suggests that distinct, intact, optically active compounds were present in freshly produced CDOM.

7. We have worked out several of the problems associated with solid phase extraction and are confident that our current technique, using both SPE and ultrafiltration, is well suited for isolating CDOM with a wide range of chemical properties. We are currently waiting for DOC concentration data in order to quantify the fraction of total DOC that is isolated with these techniques.
8. Fe affinity chromatography has given mixed results. Model ligands can be efficiently absorbed onto the column and eluted with high recoveries, but results using our natural isolates are thus far inconclusive. Further work is needed on this technique to ensure that we are able to specifically target Fe(III)-binding components of natural organic matter.

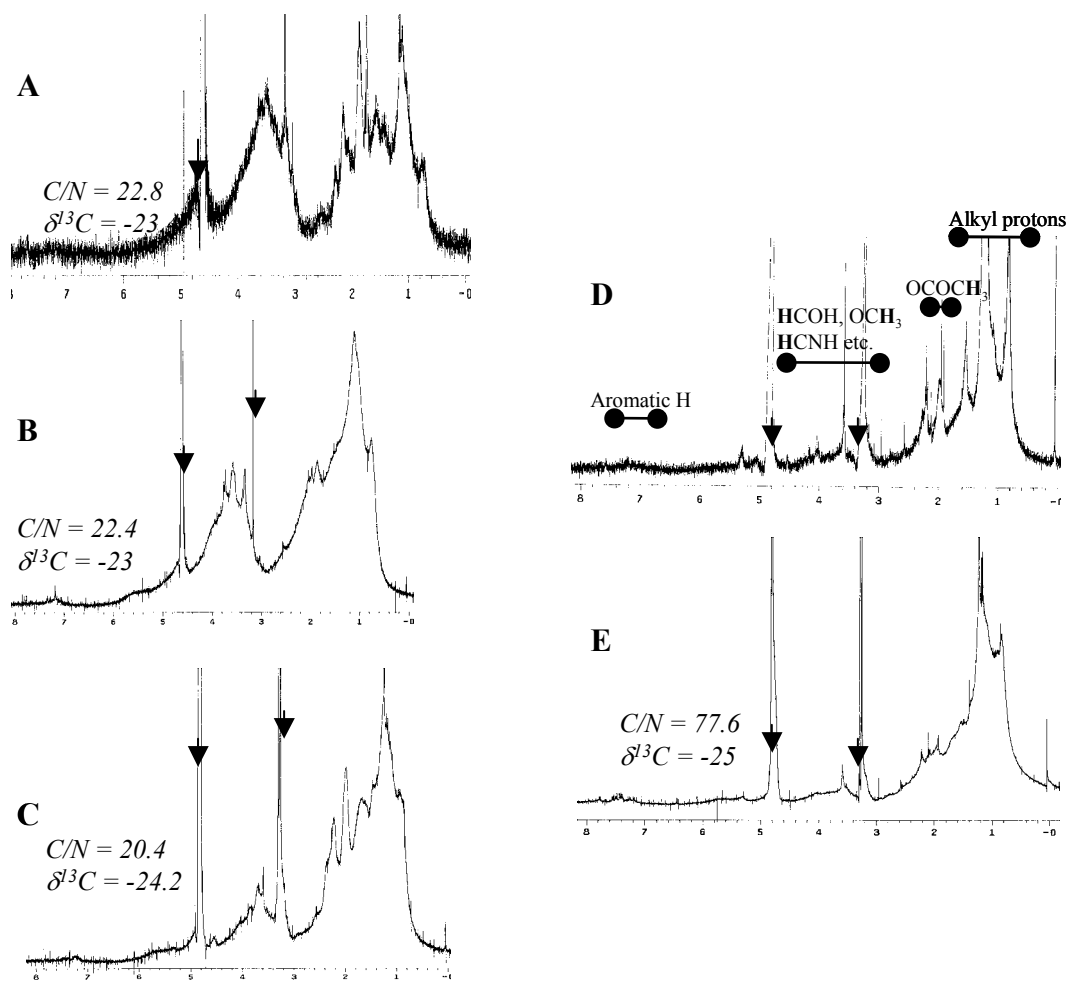


Figure 1. Proton (^1H) nuclear magnetic resonance (NMR) spectra for five different chemical fractions (A (most polar) through E (least polar)) of DOM isolated from surface seawater using a solid phase extraction resin and eluting with various solvents. Arrows on each NMR spectrum represent resonances arising from NMR solvents. Noted on NMR spectrum D are the regions/resonances that correspond to particular functional groups. Also noted on each spectrum is the C/N ratio and $\delta^{13}\text{C}$ value for each chemical fraction.

Photochemistry Experiments

9. In a photochemical experiment with a CDOM sample isolated from 1000 m in the North Central Pacific, both LMW and HMW fractions and the HMW fraction spiked with 100 nM Fe were exposed to natural sunlight for several hours. The LMW CDOM fraction showed a loss in DOC, and significant loss of absorption in specific regions of the UV-Vis spectrum (Figure 2). The HMW CDOM sample showed some decrease in absorption, and the photobleaching effect was enhanced in the Fe-spiked sample at wavelengths < 350 nm. Another photochemical experiment was conducted with a non-fractionated DOM sample collected at the SIO pier during an intense flagellate bloom. This sample was photoreactive and a loss in absorption intensity was observed relative to a dark control. The addition of Fe did not enhance photobleaching, but this could be due to the presence of relatively high Fe levels already in the sample. We plan to repeat these experiments after removal of any complexed Fe present via addition of a competitive ligand.

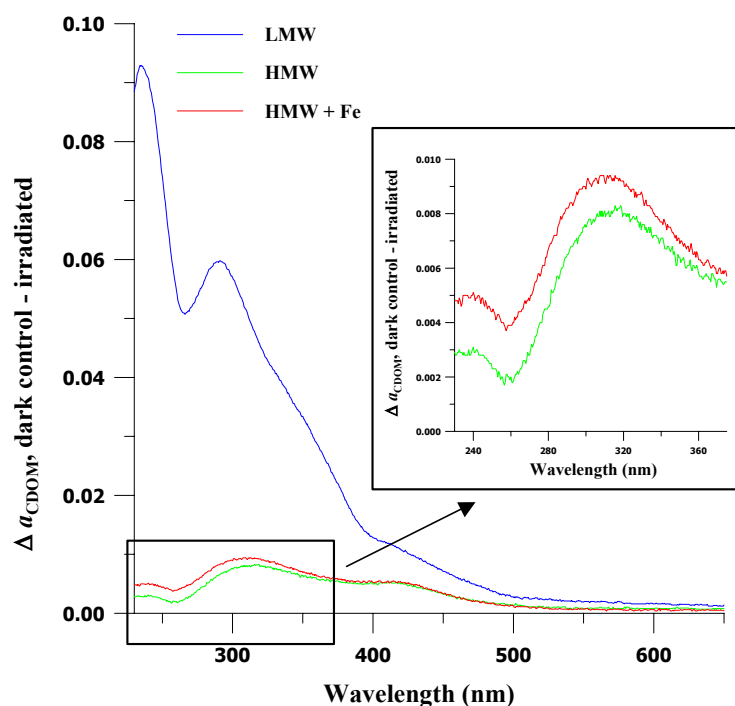


Figure 2. Effects of natural sunlight irradiation (6 hrs) and added Fe on the degradation (as measured by loss of absorbance) of low- and high-molecular weight fractions of chromophoric organic matter isolated from the deep Pacific (1000 m, station ALOHA). Results indicate significant, wavelength-specific bleaching of the low molecular weight fraction. Addition of Fe enhanced photobleaching of the high molecular weight fraction.

IMPACTS/APPLICATIONS

Our approach to CDOM characterization in this project has involved the development of novel isolation methods, which combine ultrafiltration and solid phase extraction protocols. These new techniques should be widely applicable to general studies of dissolved organic matter in aqueous environments, and these methods promise to enhance our ability to elucidate the biogeochemical

cycling of DOM (both chromophoric and non-chromophoric) in marine systems. Our data represent some of the first direct structural and photochemical studies of CDOM that is truly marine in origin. These findings support the view that optically active compounds are endogenously produced in marine systems and reveal novel structural information about these components. Initial photochemical experiments with fractionated marine CDOM from the deep North Pacific clearly indicate the potential for significant photobleaching of both low and high molecular weight components upon exposure to sunlight, and enhancement of these effects by Fe complexation. Our results are providing valuable new information about the structure and cycling of marine-derived CDOM in the oceans, adding to a database, which thus far has been dominated by studies of terrestrially derived CDOM in estuarine systems of the US east coast.

RELATED PROJECTS

Recently, LIA has obtained funding from the Lawrence Livermore National Labs (UCRP; start date October 1, 2003) to radiocarbon date the individual DOM fractions obtained as above to determine the residence time of various compounds in seawater. In particular, we are interested in determining whether the photoreactive, CDOM fraction, isolated from the deep ocean, imparts the old age (~6000 radiocarbon years) to DOM.

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