Marine Chemistry 164 (2014) 25–37

Contents lists available at ScienceDirect

Marine Chemistry

journal homepage: www.elsevier.com/locate/marchem

Colloidal size distribution of humic- and protein-like fluorescent organic matter in the northern Gulf of Mexico

Björn Stolpe *, Zhengzhen Zhou [1], Laodong Guo [1], Alan M. Shiller

Department of Marine Science, University of Southern Mississippi, 1020 Balch Blvd., Stennis Space Center, MS 39529, USA

ARTICLE INFO

Article history:
Received 14 November 2013
Received in revised form 9 May 2014
Accepted 18 May 2014
Available online 3 June 2014

Keywords:
Colloids
Fluorescence
Humic
Protein
Field-flow fractionation
Gulf of Mexico
Mississippi

ABSTRACT

The colloidal size distributions (0.5–400 nm) of humic-like and protein-like fluorescent and UV-absorbing marine dissolved organic matter (DOM) have been determined using flow field-flow fractionation (FlFFF) coupled to UV-absorbance and fluorescence detectors. Surface seawater samples were taken monthly between November 2008 and September 2009, from eight stations along a transect across the Mississippi Sound and Mississippi Bight in the northern Gulf of Mexico. DOM was also isolated from cultures of two phytoplankton species. The concentration of dissolved organic carbon (DOC) in the study area was largely controlled by river inputs, but the specific UV-absorbance and fluorescence index indicated that the importance of marine DOM increased from nearshore to offshore stations. In the seawater samples, only a small fraction (3–16%) of the 'humic-like' fluorescent DOM (Ex/Em wavelengths at 240/440 nm, 310/400 nm, 320/440 nm and 350/450 nm) was found in the colloidal size range (>0.5 nm), mainly associated with small colloids (mean hydrodynamic diameter 2–3 nm). For the 'protein-like fluorescent DOM' (Ex/Em at 275/305 nm and 275/340 nm) a larger fraction (21–100%) was found in the colloidal size range, including both small (2–3 nm) and larger (mean hydrodynamic diameter 6–7 nm, ~75 nm, and >400 nm) colloids. A culture of the diatom *Chaetoceros muelleri* produced mainly humic-like fluorescent DOM, while the fluorescent DOM produced by the dinoflagellate *Prorocentrum sp.* was mostly protein-like. In both plankton cultures, the protein-like DOM was largely associated with colloids around 6 nm, 12–14 nm and larger, while only a small fraction of humic-like DOM was found in the colloidal size range. After five months of starving and aging of the cultures, both the humic-like and protein-like DOM became increasingly associated with small colloids having a mean hydrodynamic diameter of 2–4 nm. We hypothesize that the larger (6–7 nm, 12–14 nm, ~75 nm and >400 nm) protein-like colloids in the seawater samples and phytoplankton cultures were freshly produced by phytoplankton or bacteria. The smaller (2–4 nm) colloids in the aging phytoplankton cultures probably represented phytoplankton-derived organic material that had been transformed by microbial degradation, while the small (2–3 nm) humic-like colloids in the seawater samples were most likely dominated by fulvic acids from terrestrial sources.

1. Introduction

Marine dissolved organic matter (DOM) is one of the largest reduced carbon reservoirs on earth, and plays a central role in marine ecosystems and the global carbon cycle (Hedges, 2002). Because DOM in the coastal ocean is a complex mixture of compounds from both marine and terrestrial sources, and varies in molecular size, age and lability (Amon and Benner, 1996; Guo et al., 1996), our understanding of its cycling in the ocean requires improved characterization methods. Fluorescence spectroscopy has been increasingly used as a characterization technique for the light-absorbing fraction of natural DOM, owing to its high sensitivity, ease of use and high selectivity for organic composition (Coble, 2007; Fellman et al., 2010). Fluorescence excitation and emission spectra can be combined into three-dimensional matrices (3D EEM), in which fluorescence maxima at certain excitation/emission wavelengths (fluorophores) have been related to specific organic compounds (Coble et al., 1990; Mopper and Schultz, 1993; Stedmon et al., 2003). The fluorophores of natural DOM have been broadly classified as either 'humic-like', having emission maxima in the 370–500 nm range, or 'protein-like', with emission maxima in the 300–370 nm range (Coble, 2007; Fellman et al., 2010). Sub-categories like 'terrestrial' and 'marine' humic-like as well as 'tyrosine' and 'tryptophan' protein-like fluorophores have been further identified (Coble et al., 1990; De Souza Sierra et al., 1994; Mopper and Schultz, 1993). Protein and humic-like fluorescence often show contrasting distributions and variations in marine environments. In-depth profiles from the open ocean, protein-like fluorescence has shown distinct surface maxima, indicating

* Corresponding author at: AkzoNobel Pulp & Performance Chemicals, 445 80 Bohus, Sweden.
E-mail addresses: bjorn.stolpe@gmail.com (B. Stolpe), zhou9@uwm.edu (Z. Zhou), guol@uwm.edu (L. Guo), alan.shiller@usm.edu (A.M. Shiller).
[1] Current address: School of Freshwater Sciences, University of Wisconsin-Milwaukee, 600 E. Greenfield Ave. Milwaukee, WI 53204, USA.

http://dx.doi.org/10.1016/j.marchem.2014.05.007
0304-4203/

that it is associated with recently photosynthesized organic matter (Jørgensen et al., 2011; Wedborg et al., 2007; Yamashita et al., 2010). Humic-like fluorescence, in contrast, has been found to correlate with the major nutrients and apparent oxygen utilization, indicating that it is associated with DOM formed by the microbial mineralization of sinking organic particles (Chen and Bada, 1992; Hayase and Shinozuka, 1995; Yamashita et al., 2007). In coastal environments, humic-like fluorescence usually correlates inversely with the salinity (Kowalczuk et al., 2009; Murphy et al., 2008; Yamashita et al., 2008), showing that it is mainly associated with freshwater DOM. Protein-like fluorescence on the other hand shows either a slower decrease with the salinity (Guo et al., 2011; Kowalczuk et al., 2009; Yamashita and Tanoue, 2004a) or a mid-salinity maximum (Parlanti et al., 2000; Yamashita et al., 2008), and high concentrations during phytoplankton blooms (Etheridge and Roesler, 2004; Yamashita and Tanoue, 2003), indicating an additional source from in situ phytoplankton production. In laboratory incubations and mesocosm experiments, the protein-like fluorescence has been found to increase during phytoplankton production (Stedmon and Markager, 2005) and during the initial stage of phytoplankton decay (Boyd and Osburn, 2004; Moran et al., 2000), while the humic-like fluorescence only increased after long-term dark incubations (Parlanti et al., 2000; Rochelle-Newall and Fisher, 2002), indicating that it was associated with the DOM remaining after microbial degradation of phytoplankton organic matter. These observations have led to the hypothesis that protein- and humic-like fluorescence could be used as specific tracers for "new" autochthonous and "old" allochthonous DOM, respectively (Coble, 2007; Fellman et al., 2010; Mopper et al., 1996).

There are strong indications that the age, source and digenetic state of DOM vary with molecular size (Amon and Benner, 1996; Hama et al., 2004; Santschi et al., 1995). Nevertheless, there have been relatively few applications of fluorescence spectroscopy to characterize DOM in different size fractions, and the results have been contradictory. Studies using crossflow ultrafiltration have found humic-like fluorescent DOM in coastal and offshore marine waters to be mainly distributed in the <1 kDa fraction (Mopper et al., 1996), whereas protein-like fluorescent DOM has been shown to be slightly shifted towards larger size fractions although mainly found in the <5 kDa range (Yamashita and Tanoue, 2004b). However, the separation of DOM into a few size fractions that is achieved by ultrafiltration can only give limited information about the size distributions of the different types of fluorescent DOM (Guo and Santschi, 2007). Flow field-flow fractionation (FlFFF) on the other hand is a chromatography-like elution technique that separates colloids (1–1000 nm) on a continuous scale based on their size-dependent ability to diffuse against a flow of liquid in an open channel (Baalousha et al., 2011; Giddings, 1993). FlFFF with on-line detectors can reveal the size distributions of discrete colloid-types, information which has been used to investigate the sources and dynamics of colloids in aquatic environments (Boehme and Wells, 2006; Stolpe et al., 2013a; Stolpe et al., 2013b). Until now, marine applications of FlFFF have mainly focused on humic-like fluorescent colloids (Hassellöv, 2005; Stolpe and Hassellöv, 2010; Zanardi-Lamardo et al., 2002), while protein-like fluorescence has either been measured only 'off-line' in a few size fractions collected from the FlFFF effluent (Boehme and Wells, 2006), or by on-line detection in a very limited number of samples (Stolpe et al., 2010).

In the present study, we have used FlFFF with on-line UV-absorbance and fluorescence detectors, together with 3D fluorescence spectroscopy, to determine the continuous colloidal size distributions (0.5–400 nm) of humic- and protein-like fluorescent DOM, and their seasonal and geographical variations in both the total dissolved (<700 nm) and colloidal fractions in surface seawater from the northern Gulf of Mexico. DOM isolated from cultures of two different phytoplankton species was also characterized for comparisons with natural DOM samples. Our study is one of very few to show the continuous colloidal size distribution of both humic-like and protein-like fluorescent DOM in a marine environment. The sampling was carried out prior to the Deepwater Horizon oil spill which affected the fluorescent signatures in the water column of our study area (Zhou and Guo, 2012; Zhou et al., 2013a; Zhou et al., 2013b) and our data presented here should thus represent baseline fluorescence signatures before the oil spill.

2 . Materials and methods

2.1. Study area and sampling

Water samples were collected in the Mississippi Sound and Mississippi Bight in the northern Gulf of Mexico (Fig. 1). The Mississippi Sound is adjacent to St. Louis Bay, and is separated from the Mississippi Bight by a chain of barrier islands. The study area in the northern Gulf of Mexico is a dynamic coastal environment (Conmy et al., 2004; Guo et al., 2009; Lohrenz et al., 2008) influenced by both intensive primary production and freshwater inputs from the Mississippi River to the southwest, the Pearl River to the northwest, and to some extent smaller rivers such as the Wolf and Jourdan Rivers through St. Louis Bay in the north (Fig. 1). Surface water samples were taken on-board the R/V Lemoyne, based at the University of Southern Mississippi (USM), during seven different sampling trips between 20 November 2008 and 30 September 2009. Eight stations were occupied during each sampling on a 106 km transect from the mouth of the St. Louis Bay (30.19°N; 89.18°W) to the Gulf of Mexico (30.02°N; 88.89°W, Fig. 1).

The water depth varied from 4 m at stations 1 and 3 to ~20 m at station 8. The surface salinity, chlorophyll-*a* fluorescence (Ex/Em at 470/695 nm) and chromophoric organic matter fluorescence (CDOM fluorescence, Ex/Em at 370/460 nm) were measured in situ during sampling, using a sensor probe (ECO 3, WetLabs). The samples were taken by hand, in acid-rinsed polyethylene containers, about 10 cm below the surface, and were transported in a cooler to the laboratory at the Stennis Space Center, Mississippi, where they were filtered through combusted 0.7 μm glass fiber filters within 5 h of the sampling.

2.2. Plankton cultures

The dinoflagellate *Prorocentrum sp.* and the diatom *Chaetoceros muelleri* (CCMP1316, Provasoli-Guillard Culture Collection, Bigelow Laboratories for Ocean Sciences) were cultivated in synthetic 34 salinity seawater, kept at 8 °C and diurnal light cycle, and fertilized with EDTA-free plankton nutrient solution. After the exponential growth phase, the cultures were starved and aged for five months, without fertilization but with the 8 °C temperature and diurnal light cycle maintained. DOM was isolated from the cultures by filtration through 0.45 μm polyvinylidene fluoride filters (Durapore, Millipore) during the exponential growth phase and after the 5 months aging.

2.3. Measurements of dissolved organic carbon, UV-absorbance and fluorescence

DOC-concentrations were measured by the high-temperature combustion method using a Shimadzu TOC-V analyzer (Zhou et al., 2013a). The samples were acidified with concentrated HCl to pH < 2 before analysis. The analytical precision was <2% in terms of coefficient of variation. Consensus reference material (CRM), a deep-sea water reference material from the University of Miami (Miami, FL), was used as an external DOC standard during sample analysis (Zhou et al., 2013a).

The UV-absorbance spectra were measured in the 200–800 nm wavelength range, using a photo-diode array spectrophotometer (Agilent 8453). The specific UV-absorbance ($SUVA_{254}$) was calculated by normalizing the decadal UV-absorbance at 254 nm by the DOC concentration (Weishaar et al., 2003). The 'spectral slope' ($S_{290–400}$) was calculated from the linear slope of the natural logarithm-transformed spectra in the 290–400 nm wavelength range (Bricaud et al., 1981).