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Composition of a protein-like fluorophore of dissolved organic matter in coastal wetland and estuarine ecosystems

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ABSTRACT

This study demonstrates the compositional heterogeneity of a protein-like fluorescence emission signal (T-peak; excitation/emission maximum at 280/325 nm) of dissolved organic matter (DOM) samples collected from subtropical river and estuarine environments. Natural water samples were collected from the Florida Coastal Everglades ecosystem. The samples were ultrafiltered and excitation–emission fluorescence matrices were obtained. The T-peak intensity correlated positively with N concentration of the ultrafiltered DOM solution (UDON), although, the low correlation coefficient ($r^2 = 0.140$, $p < 0.05$) suggested the coexistence of proteins with other classes of compounds in the T-peak. As such, the T-peak was unbundled on size exclusion chromatography. The elution curves showed that the T-peak was composed of two compounds with distinct molecular weights (MW) with nominal MWs of about $>5 \times 10^4$ (T_1) and $\sim 7.6 \times 10^3$ (T_2) and with varying relative abundance among samples. The T_1-peak intensity correlated strongly with [UDON] ($r^2 = 0.516$, $p < 0.001$), while T_2-peak did not, which suggested that the T-peak is composed of a mixture of compounds with different chemical structures and ecological roles, namely proteinaceous materials and presumably phenolic moieties in humic-like substances. Natural source of the latter may include polyphenols leached from senescent plant materials, which are important precursors of humic substances. This idea is supported by the fact that polyphenols, such as gallic acid, an important constituent of hydrolysable tannins, and condensed tannins extracted from red mangrove (*Rhizophora mangle*) leaves exhibited the fluorescence peak in the close vicinity of the T-peak (260/346 and 275/313 nm, respectively). Based on this study the application of the T-peak as a proxy for [DON] in natural waters may have limitations in coastal zones with significant terrestrial DOM input.

1. Introduction

Dissolved organic nitrogen (DON) is an important source of N for microorganisms in many aquatic ecosystems (Bushaw et al., 1996). This is particularly the case in oligotrophic surface water where DON can comprise a major portion of total dissolved N (TDN) (Boyer et al., 1997; Bronk, 2002; Boyer, 2006). As such, understanding the source, bioavailability, and environmental dynamics of DON is crucial to better understand biogeochemical processes in aquatic environments.

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DON concentration (DON) in aquatic environments is generally determined as the difference between [TDN] and [DIN] (dissolved inorganic N; NH_4^++NO_3^-/NO_2^-). However, the development of alternative methods which are sensitive and can handle high sample throughput at low cost are awaited for the assessment of DON dynamics in aquatic environments. Fluorescence spectroscopy, which meets the above mentioned operational requirements, has been shown to be useful in studying the source and composition of chromophoric dissolved organic matter (CDOM) in aquatic environments (Kowalczuk et al., 2003, Stedmon et al., 2003, Maie et al., 2006c). This technique can detect proteinaceous materials, a major form of DON (Coble, 1996; McCarthy et al., 1997; Yamashita and Tanoue, 2003; Maie et al., 2006b). As such, fluorescence spectroscopy might serve as an alternative method to assess concentrations of proteinaceous materials and thereby DON in natural water. Yamashita and Tanoue (2003), for example, showed a positive correlation between protein-like fluorescence intensity (tyrosine and tryptophan peaks or peaks B and T, respectively) and the total hydrolysable amino acid concentration of coastal marine waters. Recognizing the importance of the association between dissolved organic matter (DOM) composition and bioavailability, Cammack et al. (2004) showed a positive relationship between the T-peak intensities and the rates of heterotrophic bacterial metabolism in southern Québec lakes. Several researchers also used protein-like fluorescence to estimate the relative abundance of proteinaceous compounds in CDOM (Lu et al., 2003; Reynolds, 2003; Jaffé et al., 2004). However, other fluorophores can overlap the T-peak (Mayer et al., 1999; Reynolds, 2003; Yamashita and Tanoue, 2003; 2004), and therefore a closer examination of the molecular composition of this peak is necessary to evaluate the applicability of this proxy for assessing [DON] in coastal/estuarine ecosystems. As such, we applied size exclusion chromatography (SEC) to unbundle the T-peak constituents, and investigated the relationship between the fluorescence intensity of resolved peaks and DON concentration. Furthermore, the possible source of a DON-unrelated T-peak was also discussed. Ultrafiltered DOM (UDOM) samples were used in this study to achieve DOM concentration high enough to allow detection with a fluorescence detector on an HPLC system.

2. Materials and methods

2.1. Sample collection and preparation

Natural water samples were collected at the end of the dry (March–April) and the wet (September–October) seasons of 2002 and 2003 along two major freshwater to marine transects within the Florida Coastal Everglades (FCE), namely, Shark River Slough (SRS) and Taylor Slough (TS) including also Florida Bay (FB), and are part of the FCE-Long Term Ecological Research (LTER) program (Fig. 1). SRS sites S1–S3 and TS sites T1–T3 are freshwater marsh sites dominated by emergent vegetation such as *Cladium jamaicense* (sawgrass) and *Eleocharis cellulosa* (spikerush) with high abundance of calcareous periphyton. Differences in hydroperiod between SRS and TS result in the accumulation of peat and marl at these sites,

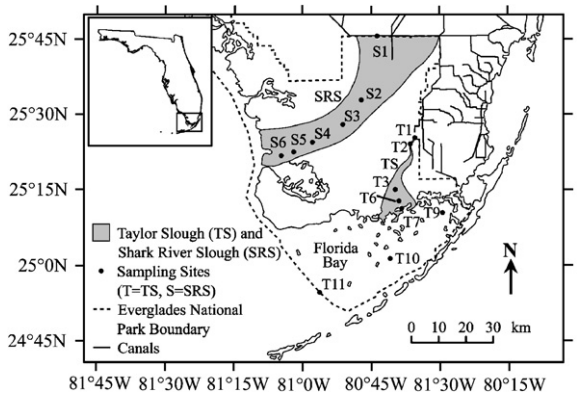

Fig. 1 – Map of sampling locations.

respectively. Sites S4–S6 and T6 and T7 are dominated by mangroves while T9 to T10 are shallow estuarine sites dominated by seagrass communities. Detailed descriptions of the sampling stations can be found on the FCE-LTER website 〈http://fcelter.fiu.edu〉 and elsewhere (Childers et al., 2006).

Sixteen to fifty liters of surface water was collected in Nalgene® low density polyethylene carboys (Nalge Nunc International), transported to the laboratory, and pre-filtered through pre-combusted (450 °C for 4 h) GF/F glass fiber filters (0.7 μm; Whatman International Ltd., Maidstone, England) and 0.22 μm Durapore® Pellicon 2 Mini membranes (Millipore Co., Billerica, MA, USA) successively within 2 d after collection. Filtered samples were stored in a refrigerated room (< 4 °C) in the dark for a maximum of 1 week. Ultrafiltered dissolved organic matter (1 kDa < UDOM < 0.2 μm) in the water samples was concentrated using a Pellicon 2 Mini tangential flow ultrafiltration system (Millipore) and freeze dried (Maie et al., 2005, 2006b). Before fluorescence analysis, samples were re-hydrated with 0.05 M Tris(hydroxymethyl)aminomethane (THAM) (adjusted to pH at 7.0 with phosphoric acid) to a concentration of 20 mgC L^{-1} for FB samples (T9, T10, and T11) and 5 mgC L^{-1} for all other samples.

2.2. Phenolic compounds

Gallic acid (3,4,5-trihydroxybenzoic acid) was purchased from Sigma-Aldrich (St. Louis, MO). Condensed tannins were extracted from senescent yellow leaves of the red mangrove (*Rhizophora mangle*) and purified according to Maie et al. (2003). Mangrove leaves were handpicked from trees along the mangrove fringe of Northeast FB (T_7 = 25°19N, −80°64W).

2.3. Fluorescence excitation emission matrices (EEM)

A series of emission fluorescence spectra of the samples were obtained at a ratio mode (emission signal-to-excitation lamp output) from λ+10 nm to λ+250 nm at 1 nm intervals, where λ is the excitation wavelength. The excitation wavelength was scanned from 260 to 455 nm every 5 nm, in a 1 cm quartz fluorescence cell at room temperature (22 °C), using a JY-Horiba Fluoromax-3 spectrofluorometer (Horiba Jobin Yvon Inc. Edison, NJ. USA). Fluorescence values were corrected for