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Interactions between protein-like and humic-like components in dissolved organic matter revealed by fluorescence quenching

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ABSTRACT

Numerous reports have documented the interactions of fluorescent dissolved organic matter (FDOM) with other compounds such as metals and trace contaminants by characterizing the fluorescence quenching of the FDOM components. As FDOM is composed of numerous components, inter-component interactions can potentially take place. This study investigated the interactions between protein-like and humic-like components in FDOM using titration experiments and end-member mixing tests. We found that the co-occurrence of protein-like and humic-like components in FDOM samples resulted in an overlap behavior between their fluorescence peaks related to inter-component interactions. Our results suggest that the fluorescence of the protein-like components could be greatly quenched by the humic-like components in the FDOM samples, e.g., the humic-like components from Suwannee River and Nordic Reservoir FDOM yielded significant quenching effect for tyrosine (52% and 46%, respectively) and tryptophan (35% and 36%, respectively) in the titration experiments. The fluorescence of the humic-like components, however, was not impacted by the protein-like components. With the help of complexation modeling, we found that the binding capability between protein-like and humic-like components was dependent on their sources. This study could enhance our current knowledge on the role of FDOM in water and it is also important to the monitoring of FDOM by fluorescence spectroscopy.

1. Introduction

Dissolved organic matter (DOM) plays an important role in both natural waters and engineered systems (e.g., water treatment facilities) (Coble, 2007; Henderson et al., 2009; Ishii and Boyer, 2012). Because of the complexity of DOM (Leenheer and Croue, 2003), accurate and informative monitoring is a challenge when attempting to increase the understanding of DOM characteristics (Henderson et al., 2009; Ishii and Boyer, 2012). In recent years, advanced analytical techniques, such as Fourier-transform ion cyclotron mass

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spectrometry (FT-ICR MS) (Tfaily et al., 2013; Tremblay et al., 2007; Zhang et al., 2014), multidimensional nuclear magnetic resonance (NMR) spectroscopy (Pautler et al., 2012; Tfaily et al., 2013) and excitation-emission matrix (EEM) fluorescence spectroscopy (Carstea et al., 2014; Pautler et al., 2012; Tfaily et al., 2013; Tremblay et al., 2007), have been actively used for the characterization of DOM. Monitoring of EEM fluorescence has been cited as a powerful technique for the characterization of FDOM (Nebbioso and Piccolo, 2013). Modeling of parallel factor (PARAFAC) analysis, which can decompose the EEM spectrum into independent fluorophore groups (Stedmon and Bro, 2008), has substantially improved the capacity of EEM in characterizing FDOM (Nebbioso and Piccolo, 2013; Stedmon and Bro, 2008). As such, EEM measurements and PARAFAC analysis have been increasingly used to explore organic matter in water (e.g., freshwaters (Jaffé et al., 2014; Mladenov et al., 2011; Murphy et al., 2008) and human-impacted waters (Baker, 2001; Goldman et al., 2012; Meng et al., 2013; Yang et al., 2014a; Zhang et al., 2011)). However, the EEM measurements have not fully considered the potential impacts of the interactions between fluorophore groups on the measured data.

Aquatic DOM contains numerous chemical molecules with a high content of oxygenated reactive functional groups, such as carboxylic, phenolic and alcoholic groups (Plaza et al., 2006). The presence of these groups enables DOM to have high complexation capacities (Plaza et al., 2006; Tipping, 2002), such as complexation with metals (Chappaz and Curtis, 2013; Riedel et al., 2012, 2013; Yamashita and Jaffe, 2008; Yan and Korshin, 2014) and pharmaceuticals (Hernandez-Ruiz et al., 2012). The binding propensity of FDOM or DOM with other compounds is usually characterized by changes in optical properties (Chappaz and Curtis, 2013; Plaza et al., 2006; Yamashita and Jaffe, 2008; Yan et al., 2013). It has been demonstrated that the combined use of EEM measurements and PARAFAC analysis was sensitive enough to determine the binding capacities of FDOM, which were often revealed by fluorescence quenching (Wu et al., 2011; Yamashita and Jaffe, 2008). Previous studies were mostly focused on how and to what extent other compounds (e.g., heavy metals and pharmaceuticals) interacted with FDOM. However, we should note that FDOM itself is composed of numerous molecular assemblies (Peuravuori and Pihlaja, 2004; Piccolo, 2001; Romera-Castillo et al., 2014) that can potentially take part in inter-molecule or inter-component interactions. Further investigation of FDOM inter-component interactions is of high interest for the understanding of FDOM transport in water ecosystem and the monitoring of fluorophores by fluorescence spectroscopy.

Human activities have seriously impacted the concentrations, compositions and characteristics of DOM in urbanized rivers, lakes and coastal oceans (Baker, 2001; Goldman et al., 2012; Meng et al., 2013; Mladenov et al., 2011; Mostofa et al., 2013; Murphy et al., 2008; Zhang et al., 2011). The discharge of treated wastewater is one of the most important anthropogenic inputs to DOM in water (Baker, 2001; Goldman et al., 2012). Note that effluent organic matter (EfOM) has different compositions and characteristics from naturally occurring organic matter (NOM) (Goldman et al., 2012; Neale et al., 2012; Yang et al., 2014b), e.g., EfOM is composed of soluble microbial products and non-biodegraded wastes (Meng et al., 2009) and has a higher protein abundance than NOM (NOM is often dominated by humic-like substances). Thus, wastewater-impacted FDOM in urbanized rivers could appear as a significant Peak T (tryptophan-like fluorophores) and/or a Peak B (tyrosine-like fluorophores) at lower Ex/Em wavelengths in the EEM spectra (Baker, 2001; Henderson et al., 2009; Meng et al., 2013). Generally, the mixing behavior of FDOM with different sources could give rise to interactions between their end-members pools (Yang and Hur, 2014), leading to changes in their optical properties (Hur et al., 2006; Myat et al., 2013). We expect that different fluorophore groups in wastewater-impacted FDOM, such as protein-like and humic-like substances, will likely interact with each other (Myat et al., 2014); however; few studies have thus far been performed to assess the potential for fluorescence quenching within FDOM components.

The aim of this study is to reveal the fluorescence quenching behavior within FDOM components and to reveal to what extent they interact with each other. Interactions between protein-like components (tyrosine and tryptophan) and humic-like components (two NOM samples purchased from the International Humic Substances Society (IHSS)) were ascertained using titration experiments. This study is novel in revealing the naturally occurring fluorescence quenching within the FDOM components.

2. Materials & methods

2.1. DOM source materials

Suwannee River NOM (SRNOM, 2R101N) and Nordic Reservoir NOM (NRNOM, 1R108N) were obtained from the IHSS. L-Tryptophan (99.0–101.0%), L-Tyrosine (>99%) and fatty-acid-free bovine serum Albumin (BSA) were obtained from Sigma. Solutions of the two NOM samples (50 mg/L), tryptophan (5 mg/L), tyrosine (5 mg/L) and BSA (50 mg/L) were prepared using Milli-Q water. Large particles as a result of incomplete solubilization in the NOM solutions were removed by filtering with pre-rinsed membranes (0.22 μm, PVDF, Millipore, USA). EfOM samples were collected from the effluent (after the final disinfection using the chlorination method) of a local wastewater treatment facility, which employed anaerobic-anoxic-oxic (A^2/O) process treating domestic wastewater. The samples were stored in acid-cleaned plastic containers, and transport to our laboratory within 1 h. Then, they were stored at 4 °C until needed. The sampling campaign was conducted on morning (7:30), noon (11:30) and evening (21:00) of three different days. The EfOM sample used in this study was the mixture of these samplings. The dissolved organic carbon (DOC), total nitrogen (TN), and total phosphorous (TP) of the samples were determined to be 12.6 mg-C/L, 1.5 mg-N/L, and 0.24 mg-P/L, respectively, on average.

2.2. Fluorescence titration

Concentrations of amino acids in natural waters are often quit low, e.g., concentrations of tryptophan and tyrosine in a bay were determined to be about 0.8 μg/L and 3.1 μg/L, respectively (Yamashita and Tanoue, 2003b). In comparison, the