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Combining asymmetrical flow field-flow fractionation with on- and off-line fluorescence detection to examine biodegradation of riverine dissolved and particulate organic matter

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ABSTRACT

This study demonstrated that asymmetrical flow field-flow fractionation (AF4) coupled with on-line UV and fluorescence detection (FLD) and off-line excitation-emission matrix (EEM) fluorescence spectroscopy can be employed to analyze the influence of microbial metabolic activity on the consumption and production of freshwater organic matter. With the AF4 system, organic matter is on-line enriched during a focusing/relaxation period, which is an essential process prior to separation. Size-fractionated chromophoric and fluorophoric organic materials were simultaneously monitored during the 30-min AF4 separation process. Two fractions of different sizes (dissolved organic matter (DOM) and particulate organic matter (POM)) of freshwater samples from three locations (up-, mid-, and downstream) along the Han River basin of Korea were incubated with the same inoculum for 14 days to analyze fraction-specific alterations in optical properties using AF4-UV-FLD. A comparison of AF4 fractograms obtained from pre- and post-incubation samples revealed that POM-derived DOM were more susceptible to microbial metabolic activity than was DOM. Preferential microbial consumption of protein-like DOM components concurred with enhanced peaks of chromophoric and humic-like fluorescent components, presumably formed as by-products of microbial processing. AF4-UV-FLD combined with off-line identification of microbially processed components using EEM fluorescence spectroscopy provides a powerful tool to study the relationship between microbial activity and composition as well as biodegradability of DOM and POM-derived DOM from different origins, especially for the analysis of chromophoric and fluorophoric organic matter that are consumed and produced by microbial metabolic activity. The proposed AF4 system can be applied to organic matter in freshwater samples having low concentration range (0.3–2.5 ppm of total organic carbon) without a pre-concentration procedure.

1. Introduction

Natural organic matter (NOM) consists of compounds formed through the degradation of several source materials, such as plants, animals, and other organisms, or it can originate from land use or other types of anthropogenic activities. Depending on origin, NOM has different compositions, sizes, and optical characteristics [1,2]. Due to precipitation and other natural processes, this organic matter flows into the riverine ecosystem in a suspended state and is transported by the stream of surface water. Organic matter in rivers has a broad size distribution that can be divided into two groups: dissolved organic matter (DOM) that passes through a filter with a nominal pore size ranging from 0.2 to 0.7 μm and particulate organic matter (POM) that is collected as the retentate on the filter. In a more concrete definition, DOM is composed of nano- to micrometer-sized colloidal particles suspended in a water stream, and POM-derived DOM (or P-DOM hereafter) is the organic matter attached to the suspended sediment. These organic materials play various important roles in biogeochemical processes in riverine and marine ecosystems, such as metal chelation, transportation of pollutants, governance of aquatic photochemistry, and as nutrition and energy sources for biomes [3–7]. Riverine DOM and POM represent a critical link in the global carbon cycle, respiring large amounts of organic carbon exported from terrestrial sources during transport to the oceans [8,9].

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Organic matter is classified into different types such as hydrophobicity, size, optical properties, and biodegradability [10]. Among them, aquatic organic matter is categorized by two types based on biodegradability: biodegradable organic matter (BOM), which can be degraded by microorganisms, and refractory humic substances, which are resistant to biodegradation [11–13]. Small fractions of both DOM and POM are digested by microorganisms and called biodegradable DOM and POM, respectively (BDOM & BPOM). Therefore, investigation of the biodegradability of organic matter is important in understanding the role of organic matter in the riverine ecosystem and the global carbon cycle. A number of studies have been conducted to elucidate the process of biodegradation in relation to source dependent degradation by microorganisms and accompanied changes in organic matter in terms of size, chemical composition, and optical properties [14–21]. Various analytical techniques such as capillary electrophoresis (CE), liquid chromatography, field-flow fractionation (FFF), mass spectrometry (MS), and fluorescence spectroscopy have been utilized to characterize organic matter from soil, riverine, and marine environments [22–25]. Among these techniques, fluorescence spectroscopy has been powerfully utilized to obtain important molecular information on organic matter. In particular, excitation–emission matrix (EEM) fluorescence spectroscopy has proven to be a useful tool to distinguish different types of organic matter based on fluorescence wavelengths [26] and has been widely utilized in practical applications such as evaluation of wastewater treatment [27,28]. For the determination of biodegradability of organic matter, two typical methods are utilized. Parallel factor analysis (PARAFAC) characterizes different aquatic organic matter using the statistical fluorescence profiling. The other method measures the amounts of carbon and nitrogen generated in the course of microbial respiration [12–19]. However, information about the changes in organic matter is not readily obtained from these methods. PARAFAC of organic matter has some pitfalls as it only shows the overall statistical profile according to the type of organic matter such as protein-like or humic-like, and it does not provide information on the size-dependent changes in organic matter or on the creation of different types of organic matter.

Flow field-flow fractionation (FlFFF) is an elution-based separation technique that takes place in a thin empty channel space through application of two perpendicular flow streams (one for migration of sample components and the other for cross-flow to retard sample migration) [29,30]. FlFFF takes advantage of the diffusion characteristics of particles or macromolecules in which particles with faster diffusion (or smaller Stokes' diameter) are distributed against the channel wall with a center of gravity (cg) higher than that of slower diffusion (larger particles); therefore, smaller particles elute earlier than larger particles in a flow stream with parabolic flow velocity profiles. Among variants of FlFFF, asymmetrical flow field-flow fractionation (AF4) has been widely utilized for size separation and characterization of proteins, cells, viruses, and water-soluble polymers [31–34]. In particular, FlFFF was employed to study the molecular weight distribution of natural organic matter [35,36], the relationship between elemental composition and size of natural colloids in the environment [37], and variations in fluorescence of chromophoric DOMs using off-line EEM fluorescence spectroscopy [38]. However, studies on the biodegradation of organic matter (BOM) using FlFFF are rare.

This study demonstrated that AF4 combined with on- and off-line FL detection can be utilized as a powerful tool to characterize chromophoric and fluorophoric DOM components of various size and origin from freshwater samples. The AF4 system employed in this study allows such a powerful detection owing to the on-line enrichment of low concentration sample before analysis and having an option to select the type of organic matter to analyze. Two different sized fractions (DOM and POM) of freshwater samples from three locations (up-, mid-, and downstream) along the Han River basin of Korea were incubated with the same inoculum for 14 days in order to analyze fraction-specific alterations in optical properties using AF4-UV-FLD and EEM fluorescence spectroscopy. Organic matter in water samples was on-line enriched in the AF4 channel prior to separation, and direct observation of the size and population of organic matter present in freshwater were readily achieved in order to examine microbially induced alterations in optical properties of DOM and POM-derived DOM (P-DOM). The present study focused on two specific types of fluorescent organic matter components, protein-like and humic-like, which were selectively monitored at each unique fluorescence wavelength by examining modifications in the size of organic matter from up-, mid-, and downstream river water induced by biodegradation.

2. Experimental

2.1. Sample preparation

Stream and river water samples were collected from three different locations in the Han River basin of Korea: a forested headwater stream in the Haean Basin, Yanggu (38°15′ N, 128°7′ E; hereafter called "upstream"); the Mandae stream, an agricultural stream draining the entire Haean Basin (38°16′ N, 128°9′ E; "midstream") on August 18; and a downstream reach of the Han River near a small island called Bamseom (37°32′ N, 126° 55′ E; "downstream") on August 19, 2014. Samples were collected following a small monsoon rainfall event with a total precipitation of 14 mm such that hydroclimatic conditions around the sampling dates represented weather conditions typical of the summer monsoon period. Water at a depth of 10–30 cm from the stream surface was collected in acid-cleaned amber glass bottles using a Masterflex® E/S™ portable field pump from Cole-Parmer (Vernon Hills, IL, USA), equipped with acid-cleaned silicone tubing. Samples were stored in the dark on ice until being filtered in the laboratory. Measured values of total organic carbon (TOC) of each sample are listed in Table S1 of Supplementary data.

Sample preparations are shown in the schematic chart in Fig. 1. The DOM and POM fractions were prepared by filtering water samples using a 0.2-μm Whatman® Nuclepore Polycarbonate membrane and a 0.7-μm Whatman® glass microfiber filter (grade GF/F), respectively, both from GE Healthcare Life Sciences (Piscataway, NJ, USA). Since POM contains organic molecules attached to suspended sediment, retrieval of P-DOM was accomplished by lyophilizing the filter paper with the retentate, cutting it into small pieces, and immersing them in ultrapure water to detach particles from the filter paper. A standard fulvic acid sample was prepared by dissolving Suwannee river fulvic acid standard from IHSS (International Humic Substance Society) in ultrapure water to a ~1 mg/L solution, which was then filtered through a 0.2-μm polycarbonate membrane. Since the P-DOM treatment involved GF/F filters throughout the incubation, "dummy" GF/F filters were added to the DOM and fulvic acid samples to create the same experimental conditions for all treatments. GF/F filters had been combusted at 450 °C for 2 h to remove organic materials and were then shredded into small pieces.

Inoculum, microbial species to induce biodegradation of organic matter, was prepared by filtering the downstream river water sample through a 2-μm polycarbonate membrane followed by incubating the filtrate in a rotary shaker for 7 days under aerobic conditions at 25 °C. The same amount of inoculum was added to each DOM, P-DOM, and fulvic acid sample at a concentration of 1% v/v (Fig. 1). Immediately after inoculum was added, part of the sample was filtered through a 0.2-μm polycarbonate