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Characterization of binding site heterogeneity for copper within dissolved organic matter fractions using two-dimensional correlation fluorescence spectroscopy

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A R T I C L E I N F O

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The heterogeneity of copper binding characteristics for dissolved organic matter (DOM) fractions was investigated based on the fluorescence quenching of the synchronous fluorescence spectra upon the addition of copper and two-dimensional correlation spectroscopy (2D-COS). Hydrophobic acid (HoA) and hydrophilic (Hi) fractions of two different DOM (algal and leaf litter DOM) were used for this study. For both DOM, fluorescence quenching occurred at a wider range of wavelengths for the HoA fractions compared to the Hi fractions. The combined information of the synchronous and asynchronous maps derived from 2D-COS provided a clear picture of the heterogeneous distribution of the copper binding sites within each DOM fraction, which was not readily recognized by a simple comparison of the changes in the synchronous fluorescence spectra upon the addition of copper. For the algal DOM, higher stability constants were exhibited for the HoA versus the Hi fractions. The logarithms of the stability constants ranged from 4.8 to 6.1 and from 4.5 to 5.0 for the HoA and the Hi fractions of the algal DOM, respectively, depending on the associated wavelength and the fitted models. In contrast, no distinctive difference in the binding characteristics was found between the two fractions of the leaf litter DOM. This suggests that influences of the structural and chemical properties of DOM on copper binding may differ for DOM from different sources. The relative difference of the calculated stability constants within the DOM fractions were consistent with the sequential orders interpreted from the asynchronous 2D-COS. It is expected that 2D-COS will be widely applied to other DOM studies requiring detailed information on the heterogeneous nature and subsequent effects under a range of environmental conditions.

1. Introduction

Dissolved organic matter (DOM), ubiquitous in aquatic and soil environments, is known to form strong complexes with metals, and it can control the bioavailability, toxicity and the ultimate fate of metals in the environment (Tipping, 1993). In aquatic ecosystems, DOM may be classified into either autochthonous and allochthonous sources. Autochthonous DOM originates from algae, bacteria, and macrophytes present in a water body, whereas allochthonous DOM is primarily derived from terrestrial carbon sources including plant- and soil-leached organic materials (Hur and Kim, 2009; Liu et al., 2009; Santos et al., 2009). Carboxylic and phenolic groups in DOM structures are reported to act as weak and strong binding sites for metals, respectively (Croué et al., 2003). In particular, carboxylic carbon content and aromatic structures of DOM are reported to have close relationships with metal binding capacities at a neutral pH condition (Merritt and Erich, 2003; Amery et al., 2007).

A number of analytical techniques are available to quantify the metal binding characteristics of DOM, which include anodic stripping voltammetry, ion selective electrode potentiometry, equilibrium dialysis, UV absorbance titration, and fluorescence quenching titration (FQT) (Bai et al., 2008; Park, 2009). Of those, FQT has been widely applied for simple comparisons of the metal binding characteristics for different types of DOM because of its simple, non-destructive, and versatile nature. A principal underlying assumption of the technique is that only 1:1 metal/ligand complexes are formed. The metal binding capacities and the stability constants have been typically determined based on a single pair of the excitation and the emission wavelengths for a DOM sample. However, the method does not account for the differences in metal binding characteristics arising from the heterogeneous distribution of the fluorophores in a bulk DOM (Luster et al., 1996; Yamashita and Jaffe, 2008). For example, a range of the extent of copper binding abilities was reported for different sized DOM fractions

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that were separated from one source of DOM (de Zarruk et al., 2007). Luster et al. (1996) suggested the presence of three different types of copper binding (i.e., very strong, strong and weak complexes) in a bulk DOM based on the observation of different fluorescence quenching behavior. Recently, efforts have been made toward a better understanding of the heterogeneity in the metal-DOM binding within a bulk DOM using advanced statistical tools. For example, Smith and Kramer (2000) have proposed a multiresponse method to account for all the fluorescence sites available for copper binding after they identified five different fluorescent components of a DOM using a spectral resolution technique. Yamashita and Jaffe (2008) derived eight fluorescence components from a bulk DOM using parallel factor analysis, and they presented different respective behavior of the metal binding.

Two-dimensional correlation spectroscopy (2D-COS) has been widely applied to examine the relationships between the dynamic spectral features at two different spectral variables (e.g. wavelengths) that are induced by external perturbations such as temperature, pH, and metal addition. One of the main advantages of using 2D-COS is to simplify the complex spectrum consisting of many overlapped peaks by extending peaks over the second dimension to enhance the spectral resolution (Ozaki et al., 2001; Jung and Noda, 2006). In addition, 2D-COS allows one to easily identify the sequential order of any subtle spectral change in response to external perturbations. Such powerful functions make 2D-COS very attractive for characterizing heterogeneous materials such as DOM. To our knowledge, no study in the environmental field has been conducted using 2D-COS for characterizing the heterogeneous nature of DOM. Interaction between DOM and metals may be a good example for the utilization of 2D-COS in DOM studies because the associated metal binding parameters can be determined by the fluorescence changes in DOM upon the addition of metals (Ryan and Weber, 1982; Luster et al., 1996; Plaza et al., 2006) and the FQT is easily compatible with 2D-COS. In a related work, Nakashima et al. (2008) utilized 2D-COS and fluorescence spectroscopy to present the molecular heterogeneity of humic acids.

Our previous works have demonstrated the association of the structural heterogeneity of DOM with its sources (Hur and Schlautman, 2003; Hur and Kim, 2009). In addition, one conventional way to characterize its heterogeneity is to separate a bulk DOM into two different fractions, hydrophobic (Ho) and hydrophilic (Hi) fractions, and to compare their characteristics (Leenheer and Croué, 2003; van Schaik et al., 2010). For this study, therefore, copper binding characteristics were compared using the HoA and the Hi fractions of DOM from two contrasting sources (i.e., algae and leaf litter). The objectives of this study are (1) to compare the synchronous fluorescence spectra and the fluorescence quenching behavior upon the addition of copper for the HoA and the Hi fractions of the algal and leaf litter DOM and (2) to explore the heterogeneity of copper binding affinities within the DOM fractions using 2D-COS combined with the synchronous fluorescence spectra.

2. Materials and methods

2.1. Sample preparation and quantification

The DOM sources included here were algae and leaf litter, each of which may represent autochthonous and allochthonous DOM, respectively. A suspension solution of algae was collected via the concentration of surface water from Lake Paldang (37°31′28″N, 127°16′42″E) using a plankton net. The extraction of DOM was done by periodically shaking the algae suspension several times for 6 h in a refrigerator. The background DOM, originally present in the lake, was negligible because dissolved organic carbon (DOC) concentration of the final solution was much higher than that of the original lake water (34.4 versus 1.3 mg C L^{-1}). Fallen leaves were collected from a deciduous forest area in the mount Seorak national park (38°09′58″N, 128°15′36″). The partially decomposed leaves were air-dried and cut into pieces of approximately 1 cm^2. The leaf pieces were then shredded to further reduce the size so that a greater amount of organic matter could be extracted per leaf mass (Hur et al., 2009b). The leaf litter DOM extract was prepared by mixing the dried litter sample with distilled and deionized water (DDW) at a solid-to-solution mass ratio of 1:10. The final DOM solutions (1094 mg C L^{-1}) were prepared by filtering the two extracts through a 0.2-μm pore size membrane filter (Cellulose acetate, Advantec).

A portion of the final DOM solutions was separated into the HoA and the Hi fractions using DAX-8 resin (Supelite). The pH was adjusted to 2.0 using HNO_3 before the solution was passed through the column packed with resin. The resin to volume ratio and the flow rate remained 0.05 and 1.3 mL min^{-1}, respectively. The effluent of the column was designated as the Hi fraction. The HoA fraction adsorbed on the resin was isolated by flushing 0.1 M NaOH solution through the column. The fractionation recovery of algal DOM was low, probably due to the presence of the hydrophobic neutral fraction retained on the resin (Table 1).

DOC concentrations of the DOM samples were measured using a Shimadzu V-CPH analyzer. The specific UV absorbance (SUVA) values of the samples were determined by dividing 100-fold of the UV absorbance at 254 nm by the DOC concentration by employing a UV–visible spectrophotometer (Evolution 60, Thermo Scientific) and a 1 cm quartz cuvette.

2.2. Synchronous fluorescence spectra

Synchronous fluorescence spectra of the bulk DOM and their fractions were recorded with a luminescence spectrometer (Perkin–Elmer LS-50B). Excitation and emission slits were adjusted to 10 nm and 10 nm, respectively, and the excitation wavelengths ranging from 250 to 600 nm were used with a constant offset ($\Delta\lambda = 60$ nm). The concentrations of the samples were diluted to 10.0 mg C L^{-1} for the fluorescence measurements. To limit second-order Raleigh scattering, a 290-nm cutoff filter was used for all samples. The fluorescence response to a blank solution (DDW)

Table 1
Specific UV absorbance (SUVA) and fluorescence characteristics of the algal DOM, the leaf litter DOM and their hydrophobic and hydrophilic fractions.

	Percent distribution (%)	SUVA (L (mg C)$^{-1}$ m^{-1})	%PLF	%FLF	%HLF
Algal DOM		1.03 ± 0.01[a]	42.9	52.6	4.9
HoA fraction	10.9	0.94 ± 0.01	43.1	56.3	1.0
Hi fraction	47.5	0.58 ± 0.02	44.7	53.8	1.9
Leaf litter DOM		3.07 ± 0.05	14.7	82.5	3.2
HoA fraction	74.4	3.68 ± 0.01	11.4	84.1	4.8
Hi fraction	18.3	0.97 ± 0.01	40.6	58.1	2.1

[a] Standard errors based on propagating the corresponding uncertainties of the repeated measurements.