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Binding interactions of algal-derived dissolved organic matter with metal ions

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HIGHLIGHTS

- One protein- and two humic-like components are found in *Scenedesmus acutus* exudates.
- Biological activities can produced peaks C and M.
- Visible photodegradation and microbial degradation affect algal exudate composition.
- Unlike Cd, Pb and Zn, Cu strongly binds to algogenic DOM.
- Significant differences in log*K* values are found between humic-like components.

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ABSTRACT

The nature and composition of dissolved organic matter (DOM) strongly influences its binding properties to heavy metals and thus their fate, mobility and toxicity in aquatic environments. Fluorescence spectroscopy with parallel factor analysis (PARAFAC) was used to characterize DOM exuded by the cosmopolitan freshwater green algae *Scenedesmus acutus* during early exponential growth phase. One protein-like (peak T; C2) and two humic-like components (peaks A + C and A + M, C1 and C3, respectively) were split half validated on 122 emission-excitation matrices (EEMs). Our data show that both humic-like could be associated with biological activities. Unlike Cd, Pb and Zn, Cu strongly binds to algogenic DOM with conditional stability constants (log*K*) averaging 5.26 ± 0.29 (from 4.85 to 5.36). Significant differences in log*K* values were found between humic-like PARAFAC components, indicating clear differences in the binding properties of humic-like components with copper.

1. Introduction

Dissolved organic matter (DOM) is a heterogeneous mixture of dissolved material found ubiquitously in aquatic systems. Aquatic DOM falls into two main categories: autochthonous (i.e. produced *in situ* such as algal exudates) and allochthonous DOM (i.e. pedogenic). In the authochthonous pool, algogenic DOM arises extracellularly via metabolic excretion or intracellularly due to autolysis of cells. The former is dominant during the exponential growth period whereas the dominance of the latter increases during the stationary phase of the algae system. The determination of the chemical properties of algal-derived DOM is important for understanding aquatic ecosystem functions.

DOM plays a large role in aquatic ecosystems including light attenuation and mobility, transport and fate of chemical species (Williamson et al., 1999; Mylon et al., 2003). DOM, and particularly humic and fulvic acids, influences greatly the fate, mobility and transport of metals in natural waters (Tessier and Turner, 1995). Depending on the nature of the chelating ligands, metal toxicity may be different. For example, Cu associated with lipophilic complexes is more toxic to algae (Stauber and Florence, 1987; Campbell, 1995; Croot et al., 2000) than bound with hydrophilic moieties (Koukal et al., 2007). Algogenic DOM and particularly extracellular polymeric substances (EPS; Koukal et al., 2007) have been found to increase the coagulation and sedimentation rates of colloidal material and associated metals (Wilkinson et al., 1997). The products of excretion by the algae blooms may contribute significantly the dissolved organic carbon pool and be an excellent source of ligands for metal complexation (Moffett, 1995). For example Vasconcelos et al. (2002) showed that the nature and concentration of marine phytoplankton exudates influenced metal uptake. The production of DOM following algal blooms and its impact on metal binding in rivers, streams and drinking reservoirs is relatively less studied compared to other types of DOM.

Excitation–emission matrix (EEM) fluorescence spectroscopy is a highly sensitive technique commonly used to study DOM in

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freshwater and marine ecosystems (Coble, 2007; Guéguen et al., 2011). This technique provides important information on DOM composition. For example, it is possible to distinguish between labile protein-like and humic-like DOM, two fluorescent groups reported in algal cultures (Henderson et al., 2008; Li et al., 2008; Romera-Castillo et al., 2010), depending on their excitation and emission wavelengths (Ex/Em). As an EEM can contain several thousands data points, it may be difficult to assess the dynamics of fluorescent DOM based on the traditional EEM peak-picking techniques as they can be time consuming and may not be reliable. However, the characterization of fluorescent DOM has improved with the application of a multivariate modeling approach called parallel factor analysis (PARAFAC) (Stedmon et al., 2003; Stedmon and Bro, 2008), which allows EEMs to be decomposed into individual fluorescent components. This new approach has been successfully applied to study variability in DOM composition in natural systems (e.g. Stedmon and Markager, 2005; Yamashita and Jaffé, 2008; Guéguen et al., 2011).

Fluorescence spectroscopy has also been successfully applied to investigate the DOM interactions with paramagnetic (Cu) and diamagnetic metals (Cd, Pb and Zn) (Ryan and Weber, 1982; Dudal et al., 2006; Yamashita and Jaffé, 2008; Wu et al., 2011, 2012). The change in DOM fluorescence intensity is used to detect the complexation with metal. Recent work using EEM-PARAFAC revealed that different types of DOM components (e.g. humic-like and protein-like) were associated with metal binding (Yamashita and Jaffé, 2008; Wu et al., 2011). For example, Cu(II) has been reported to quench humic- and protein-like fluorescence (Yamashita and Jaffé, 2008; Wu et al., 2011) whereas the addition of Pb(II) quenched in the protein- and fulvic-like region of municipal solid waste leachate (Wu et al., 2011). Titration of extracted humic acids with Cd(II), Cu(II), Pb(II) and Zn(II) at pH 6 resulted in a marked decrease of humic-like fluorescence (Plaza et al., 2006). Wu et al. (2012) showed that Cd^{2+} complexation was only associated with fulvic-like component (220/428 nm) at pH 6 whereas Cu^{2+} binding was found with all six PARAFAC components (fulvic-, humic- and protein-like). The application of EEM-PARAFAC modeling combined with fluorescence quenching has not yet been used to study the interactions of metals with DOM derived exclusively from freshwater algae, despite its potential to provide additional insight into the biogeochemical cycling of metals.

The objectives of this study were (1) to characterize the DOM produced during the exponential phase of the green algae *Scenedesmus acutus*, and (2) to investigate the binding properties of algal DOM for Cd, Cu, Pb and Zn using fluorescence quenching and PARAFAC.

2. Materials and methods

2.1. Algal culturing

S. acutus (strain 282) was obtained as axenic culture from the Canadian Phycological Research Centre (University of Waterloo, Canada). The inoculated medium was transferred to a Conviron, A-1000 environmental chamber (23 °C; 16:8 h light:dark). A negligible amount of UV light (below 400 nm) penetrated the culturing flasks, minimizing UV photobleaching occurring during the culture growth.

The algae were grown in COMBO medium (250–300 μS cm^{-1}, pH = 7.8; Andersen, 2005) with the animal trace component removed for 72 h. The algal cells were then separated from the medium by centrifugation at 2500 rpm for 10 min. The algal pellet (2×10^5 cells mL^{-1}; Reichert Bright-Line haemocytometer) was then re-suspended in sterile EDTA-free COMBO medium for an additional 72 h. EDTA was removed from the media during the experimental phase to avoid binding competition with DOM (Guéguen et al., 2003). Growth was not impaired by the use of EDTA-free COMBO media over the period of study. Finally, the algal culture was harvested during its early exponential period (day 6) and filtered through a pre-combusted 0.7 μm glass fiber filter (GFF Whatman). Three algal cultures were also 0.02 μm filtered (Whatman Anotop) to investigate the influence of algogenic DOM size on Cu binding. The culture media and glassware were autoclaved to minimize bacteria activity at the beginning of the culture. Unfortunately no bacterial abundance was available in the study.

2.2. Quenching titration

The exponentially growing 0.7 μm algal filtrate isolated from separate algal cultures was treated with the selected concentrations of Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+} using stock solutions prepared daily from their analytical grade salts $Cd(NO_3)_2$, $Cu(NO_3)_2$, $Pb(NO_3)_2$, $Zn(NO_3)_2$. Cu(II) quenching titration was also performed on 0.02 μm algal filtrate (Anotop 10, Whatman) isolated from separate algal cultures. The metal concentrations in the final solutions (pH 7.8; 0.1M $NaNO_3$) ranged from 2 to 100 μM. All solutions were kept at room temperature in the dark for 5 min to ensure complexation equilibrium prior to fluorescence scanning. No significant difference in binding properties was found when samples were allowed to equilibrate for 5 min or 24 h ($p < 0.05$), confirming that the reaction of DOM and metal complexation was fairly rapid and pseudo-equilibrium conditions can be achieved within 10–20 s (Lin et al., 1995; Wu et al., 2004). Fluorescence quenching experiments were also conducted with two amino acids (tyrosine and tryptophan; Fisher Scientific) and two fulvic acids Pony Lake and Suwannee River fulvic acid (PLFA and SRFA, respectively; International Humic Substances Society, USA). These extracted fulvic acids are commonly used as microbially-derived and terrestrial fulvic acids, respectively (Cory et al., 2010).

Fluorescence of the samples before and after metal titration was recorded using a Fluoromax-4 Jobin Yvon spectrofluorometer (Fig. 1). The EEM spectra were collected at excitation wavelengths 250–500 nm and emission wavelengths 300–600 nm (Guéguen et al., 2011). Spectrum of Milli-Q water (Millipore, 18.2 MΩ cm^{-1}) was recorded between each algal sample to ensure no contamination and subtracted from the sample spectra to remove most of the first and second order scatter peak. Fluorescence intensities were normalized to the Milli-Q water Raman area at excitation 350 nm measured daily (Lawaetz and Stedmon, 2009) and reported in Raman unit (r.u.). In addition to EEM spectra, emission spectra of

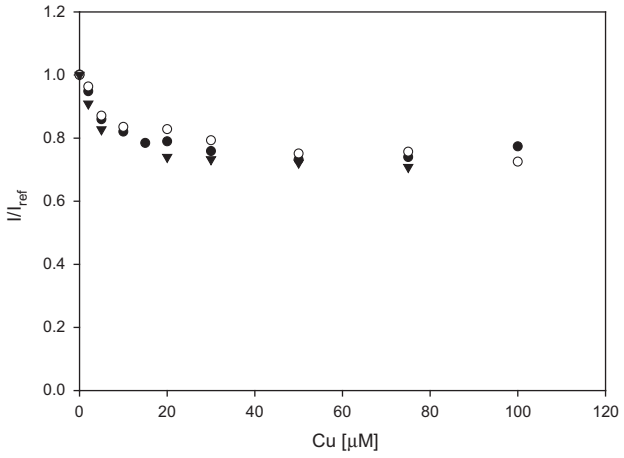

Fig. 1. Example of fluorescence quenching curves of C1 titrated with 0.01 M $Cu(NO_3)_2$ based on three separate algal cultures.