Water Research 122 (2017) 337–344

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Identifying structural characteristics of humic acid to static and dynamic fluorescence quenching of phenanthrene, 9-phenanthrol, and naphthalene

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ARTICLE INFO

Article history:
Received 23 January 2017
Received in revised form
11 May 2017
Accepted 5 June 2017
Available online 12 June 2017

Keywords:
Fluorescence dynamic quenching
Dissolved organic matter
Hydrophobic organic chemicals
Carboxyl groups

ABSTRACT

Fluorescence quenching is a sensitive and fast method to quantify the interactions between a fluorescent organic contaminant and a quencher, such as dissolved organic matter (DOM). Dynamic fluorescence quenching is resulted from molecular collision, not the real binding, and thus it complicates the binding data interpretation. On the other hand, static fluorescence quenching occurs for fluorescent contaminants of ground states, which decreases the concentration of freely dissolved contaminants. However, how a particular structure in DOM contributes to the static and dynamic fluorescence quenching of a fluorescent contaminant is still unclear, which has greatly hindered the application of fluorescence quenching technique. A humic acid (HA) extracted from sediment was chemically modified, i.e., bleaching, acid hydrolysis, and decarboxylation. HAs before and after these modifications were used in fluorescence quenching experiments for phenanthrene (PHE), 9-phenanthrol (PTR) and naphthalene (NAP). Different quenching mechanisms were observed for these chemicals depending on HA properties. For PHE and NAP, aromatic components showed static quenching, while carboxyl groups primarily showed dynamic quenching. Aromatic components and carbohydrates in HAs primarily bound (static quenching) rather than collided (dynamic quenching) with PTR. Carboxyl groups showed interactions with PTR through dynamic quenching only when carboxyl groups were on the benzene ring. Based on the results, we emphasized that dynamic quenching should be carefully excluded in fluorescence quenching studies. This line of study is important to establish a general relationship between DOM properties and static/dynamic quenching contributions.

1. Introduction

Dissolved and colloidal organic matter (DOM) plays a key role in the transport and bioavailability of contaminants, especially hydrophobic organic chemicals (HOCs) in soils by affecting the sorptive interaction between colloidal and solution phases (Gao et al., 2007; Li et al., 2015; Pan et al., 2008). Binding to DOM facilitated the transport of HOCs as well as their bioavailability in aquifers (Chabauty et al., 2016; Pontoni et al., 2015; Raber et al., 1998; Rav-Acha and Rebhun, 1992; Tremblay et al., 2005).

The interactions between DOM and HOCs are described through a carbon-normalized partition coefficients (K_{OC}) which are widely used in geochemical and ecotoxicological modeling (Shirshin et al., 2016). Owing to its fast and easy operation, fluorescence quenching is generally used in quantifying organic chemical-metal complexation (Liu et al., 1998; Merdy et al., 2009; Yaqiu et al., 2007) and the interactions between fluorescent contaminants (such as antibiotic, and polycyclic aromatic hydrocarbons) and DOM in aqueous phase (Hernandezruiz et al., 2012; Kobayashi and Sumida, 2015; Yan et al., 2016). The theory of fluorescence quenching is that the energy emission is suppressed in the presence of the quencher (DOM), which consequently results in the decrease of fluorescence intensity of the contaminant (Wang et al., 2015a). Through the remaining fluorescence intensity from the freely dissolved pollutants, we can calculate the quenched concentration and thus obtain the binding of pollutants to DOM. However, the apparent fluorescence intensity reduction (quenching) does not accurately

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http://dx.doi.org/10.1016/j.watres.2017.06.010
0043-1354/

represent the binding of contaminants to DOM. The ground state interaction of contaminants with DOM, such as complexation and binding, results in static quenching. On the other hand, the molecular collision in excited state may lead to the charge or energy complex, altering the photochemical reactions of the fluorescent contaminant (Josephy et al., 1982; Wang et al., 2001) and resulting in dynamic quenching. Static quenching reduces the freely-dissolved chemical concentration, which alters the environmental fate and risk of these chemicals, but dynamic quenching does not. It is thus essential to quantitatively distinguish dynamic quenching from the overall fluorescence quenching. Temperature-dependent quenching study or fluorescence life time measurement may be useful in evaluating if the fluorescence quenching is overwhelmed by dynamic quenching, but could not quantify its contribution (Wang et al., 2015a). In our previous work, dialysis equilibrium and fluorescence quenching methods were combined and the dynamic fluorescence quenching of phenanthrene (PHE) or ofloxacin (OFL) by DOM were successfully quantified (Wang et al., 2015a). We mostly emphasized the opposite roles of DOM hydrophobicity to the dynamic quenching of PHE and OFL. This information is very useful in investigating DOM-organic pollutant interactions, especially when fluorescence-involved method is adopted. However, how functional groups in DOM related to its quenching property is still unknown, and this line of research is generally lacking.

To better understand the quenching mechanisms as affected by structural property of DOM, a humic acid (HA) and several model compounds were used in fluorescence quenching experiment for polyaromatic hydrocarbons (PAHs). PHE, 9-phenanthrol (PTR) and naphthalene (NAP) were selected as the representative PAHs. The original HA was treated by three methods (hydrolysis, bleaching and decarboxylation) in this study. Several small organic chemicals with different functional groups were used as HA model molecules. Both dialysis equilibrium and fluorescence quenching experiments were carried out, which enabled us to quantitatively identify static and dynamic quenching. Based on the fluorescence quenching by chemically modified HAs and model chemicals with different functional groups, fluorescence quenching mechanisms could be correlated to the specific structural characteristics of the quencher. This line of study is essential to establish a general relationship between DOM properties and static/dynamic quenching contributions.

2. Methods and materials

2.1. Chemicals

PHE, PTR, and NAP were purchased from Acros Co. (Belgium) (Table 1). All three chemicals were used without further purification, and all other chemicals and solvents were better than analytical grade. These three chemicals were dissolved in methanol, then diluted to 8–80 μg/L for PHE, 25–250 μg/L for PTR and 50–200 μg/L for NAP in background solution (N_2 purged for 3 min, which was deemed sufficient to remove O_2). Methanol, as the cosolvent for these chemical solutions, was always less than 0.1% of the total volume. Background solution was prepared with 0.01 M NaCl (as ionic strength adjuster) and 200 mg/L NaN_3 (as bio-inhibitor).

2.2. Preparation of HA

The HA used in this study was extracted from Dianchi sediment, Kunming, China (24°48′N, 102°39′E) using a commonly applied alkaline extraction method (0.1 M NaOH and 0.1 M $Na_4P_2O_7$ at 1:50, w:v) as described previously (Hou et al., 2010; Kang and Xing, 2005). The obtained HA particles were structurally modified by three chemical treatments, namely, bleaching, acid hydrolysis, and decarboxylation. These treatments generated HAs with different functional groups or aromatic/aliphatic ratios. The bleaching procedure removes aromatic compositions and was performed according to the method described previously (Simpson et al., 2003). Briefly, 1 g of HA was mixed with 20 g $NaClO_2$, 20 mL acetic acid, and 200 mL deionized water. The mixture was stirred overnight, and then the supernatant was replaced by fresh treating fluid. This chemical procedure was repeated three times. Acid hydrolysis could release the loosely joined aliphatic compositions, and was carried out using 300 mL of 6 M HCl for 1 g of HA (Chefetz et al., 2002). The mixture was stirred over night, the supernatant was refreshed by HCl, and the procedure was repeated three times. Decarboxylation removes carboxyl groups, and was performed with NaOH. HA was mixed with NaOH (1: 2, w: w) and the mixture was melted at 150 °C for 5 min. All the resulted residues were centrifuged at 4000 g for 15 min, washed five times with distilled water, and then freeze-dried for further use.

To make a stock solution of HA, 0.5 g of original or treated HA particles were dissolved by raising the pH to 12 with NaOH, then shaken overnight in the dark at 25 °C. The DHA (dissolved humic acids, representing DOM) were adjusted to pH 7.0 with HCl, and then diluted with background solution to 500 mL. The DHA stock solutions were filtered using 0.45 μm filters. Molecules smaller than 3500 Da were discarded using dialysis bags (MWCO 3500, Fisher Scientific) (Pan et al., 2012) to avoid uncertainties brought by these small molecules in dialysis equilibrium experiment. Before use, the dialysis bags were washed in distilled water three times. HA solutions were enclosed in the dialysis bags and soaked in distilled water, ultrapure water, and background solution subsequently. The complete removal of small molecules was confirmed by TOC detection of the solution outside the dialysis bag. The pre-treated HA solutions inside the dialysis bags were then poured out and stored in a refrigerator (4 °C). The organic carbon contents of the prepared HA solutions were measured by a TOC analyzer (Vario TOC APSA-370, Elementar).

All the HA particles were analyzed for their elemental compositions (MicroCube, Elementar, Germany). The functional groups were examined on a diffuse reflectance fourier transform infrared (DRIFT) spectrometer (Varian 640-IR) in the transmission mode using KBr pellet (0.5 mg HA in 500 mg KBr). The spectra were collected in the range of 4000–400 cm^{-1} with 16 scans at a resolution of 8 cm^{-1}.

2.3. HAs and PHE, PTR or NAP interactions

All the HAs solutions and background solutions were purged using N_2 for 3 min to avoid dynamic quenching of chemicals by dissolved oxygen during fluorescence experiments. Depending on the solubilities of these three chemicals, sorption experiments were conducted at different initial concentration ranges, and duplicate samples were run at each concentration. The pH values of dialysis equilibrium system were adjusted to pH 7.0. For each sample, a dialysis bag was placed in a 40 mL vial with Teflon-lined screw cap, and 20 mL HA stock solution was injected (30 mg C/L) into the dialysis bag. Chemical solution and background solution were injected outside the dialysis bag, with minimum headspace to avoid re-dissolving of O_2. All the vials were continuously shaken in the dark at 25 °C until equilibrium. Seven days was long enough for apparent equilibrium according to our preliminary test. Chemical concentrations were quantified as described in the following section.

2.4. The detection of PHE, PTR and NAP

After the equilibration, solution samples were taken from