Environmental Pollution 172 (2013) 100–107

Contents lists available at SciVerse ScienceDirect

Environmental Pollution

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

Fluorescence quenching of fulvic acids by fullerene in water

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ARTICLE INFO

Article history:
Received 18 February 2012
Received in revised form
29 June 2012
Accepted 9 August 2012

Keywords:
Fullerene
Humic substance
Fluorescence
Association constant

ABSTRACT

Fullerene can be suspended in water as nanoscaled-fullerene-aggregates (nC_{60}). However, little is known about its biogeochemical cycling in natural waters. In this paper, the interactions between nC_{60} and fulvic acids were investigated using fluorescence spectroscopy and fluorescence quenching titration. The results show that the intrinsic fluorescence of fulvic acids was static quenched by adding nC_{60}. The association constants (log K) of fulvic acids and nC_{60} were estimated using a modified Ryan–Weber nonlinear model, and ranged from 6.76 to 7.41 l/mol. The log K was not significantly affected by the concentration levels of fulvic acids from 5.0 to 20.0 mg/l. The log K increased at low pH 3–5, but remained constant at high pH ranging from 5 to 11. The hydrophobic and π–π interactions were the likely primary mechanisms. The present observation will be helpful in understanding the environmental behavior of fullerene in natural aquatic ecosystems.

1. Introduction

Fullerene has shown promising applications in catalytic conversion, medical therapeutics, and nonlinear optics since its discovery in 1985 (Lyon et al., 2006). Fullerene is similar in structure to graphite, and its composition of stacked sheets that make it extremely hydrophobic, with a tendency to aggregate and deposit in polar solvents because of strong van der Waals forces. The solubility of fullerene is less than 10^{-9} mg/l in water and fullerene powder alone has no antibacterial properties (Fortner et al., 2005). However, fullerene is often required to be dispersed before potential engineering applications (Lyon et al., 2006; Yamakoshi et al., 1994). The dispersion methods commonly involve dissolving fullerene in certain kinds of solvents (e.g., dimethyl sulfoxide, polyvinylpyrrolidone, tetrahydrofuran, and toluene) that are subsequently replaced by water (Klavins and Ansone, 2010; Lyon et al., 2006). The fullerene water suspension contains nanoscaled-fullerene-aggregates (nC_{60}), whose morphological properties differ from those of dissolved fullerene in the relevant solvent (Lyon et al., 2006). The nC_{60} forms are the most environmentally relevant forms of fullerene in the event of a spill of either fullerene powder or dissolved fullerene in water (Fortner et al., 2005; Lyon et al., 2006). Furthermore, the nC_{60} forms are toxic to micro-organisms, aquatic organisms and even animal/human cell lines (Fortner et al., 2005; Lyon et al., 2006). Therefore, it is necessary to investigate the fates and behaviors of fullerene in natural environments.

Humic substances are a poly dispersed mixture of organic macromolecules with a variety of aromatic and aliphatic blocks and functional groups occurring in water, soil and sediment (Kumke et al., 1998a,b; Wu and Xing, 2009; Zafiriou et al., 1984; Zepp et al., 1987). Humic substances can be operationally separated into fulvic acid (FA; soluble at all pH values), humic acid (soluble in alkaline media and insoluble in acidic media), and humin (insoluble at all pH values), according to the water solubility (Bai et al., 2008a,b; Wu et al., 2001; Wu and Xing, 2009). FAs play key roles in affecting speciation, toxicity and transport of inorganic and organic compounds (such as Cu^{2+}, Hg^{2+}, PAHs and carbamazepine) in aquatic environments due to the strong interactions between them (Cabaniss, 2011; Fang et al., 1998; Kumke et al., 1998b; Ohno et al., 2008; Wu et al., 2004). It would be a priority to examine the interactions between nC_{60} and FAs in cases of fullerene contaminating aquatic environments. Currently, little is known about the interaction mechanisms and possible roles of FAs in controlling the environmental and biogeochemical fate of fullerene.

Molecular fluorescence quenching titration has been widely employed to provide useful information regarding the interactions between humic substances and trace contaminants since the 1980s (Bai et al., 2008a,b; Fang et al., 1998; Gauthier et al., 1986; Kumke et al., 1998a,b; Lu and Jaffe, 2001; Ryan and Weber, 1982; Wu et al., 2001, 2004). This method was also satisfactorily used to

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http://dx.doi.org/10.1016/j.envpol.2012.08.005

study the interactions between fullerene and PAHs (Datta and Mukherjee, 2006) as well as fullerene and humic substances in organic solvents (Klavins and Ansone, 2010; Manciulea et al., 2009). The interactions between humic substances and nC_{60} in water have not yet been reported.

The possible fluorescence quenching mechanisms include dynamic, static, and a combination of dynamic and static quenching (Kumke et al., 1998a; Pan et al., 2012). The dynamic quenching agent provides a non-radiative route for the loss of the excited state energy during the lifetime of an excited state of fluorescer (Pan et al., 2012). Static quenching however is a process where non-fluorescent complexes are formed. Both mechanisms were reported for different compounds quenched by fullerene (Datta and Mukherjee, 2006; Klavins and Ansone, 2010; Manciulea et al., 2009). For example, dynamic quenching is the main mechanism involved when anthracene is quenched by fullerene (Datta and Mukherjee, 2006), while the static quenching process is involved for humic substances quenched by fullerene in organic mediums (Klavins and Ansone, 2010). Therefore, the discussion on the quenching mechanism involved in water is still open. For mono-dispersal systems, the linear Stern–Volmer plot could easily be indicative of a single mechanism of fluorophores with equal accessibility to the quencher, and a combination of two quenching mechanisms typically produces a nonlinear plot (Bai et al., 2008a,b; Klavins and Ansone, 2010). Diffusion rates and dynamic collision rates increase with increasing temperature. In contrast, complex formation strength tends to decrease with increasing temperature. Thus, fluorescence quenching increases under dynamic quenching, but decreases under static quenching as temperature increases. The temperature-dependant method can simply be used to investigate the quenching mechanisms, by comparing the quenching extent at various temperatures. Dynamic quenching reduces the average lifetime of the fluorophore while static quenching does not have the same effect. Therefore, the fluorescence lifetime measurement is the most commonly used method to identify the quenching mechanism (Marwani et al., 2007, 2009). Thus, the quenching mechanisms can be confirmed by: (a) exploring the curvature with a Stern–Volmer plot, (b) comparing the association constant with an extremely efficient quencher, (c) investigating temperature dependence of fluorescence titration, and (d) fluorescence lifetime measurement (Bai et al., 2008a,b; Marwani et al., 2007, 2009). Because of the complicated composition and structure of humic substances, as well as the unknown process of their interactions with nC_{60}, all four methods were performed to investigate the main quenching mechanisms.

The objective of the present study was to investigate the interactions between FAs and nC_{60} and its affecting factors, e.g., FA concentration levels, pH values and temperatures. Using the fluorescence quenching method, temperature-dependent experiments, and fluorescence lifetime measurements, association constants between FAs and nC_{60} were carried out and estimated, and the interaction mechanisms between fulvic acid and nC_{60} are discussed.

2. Materials and methods

2.1. Reagents and materials

Fullerene with a purity of 99.9% was obtained from Puyang Yongxin Fullerene Technology Co., Ltd. (Puyang City, China). The nC_{60} suspension was produced using the method recommended by Andrievsky et al. (1995) and Lyon et al. (2006). Using this method, about 5 ml of 1.0 g/l fullerene in toluene was added to about 500 ml pure water. The layered mixture was sonicated with a sonifier cell disruptor (Ultrasonic Processor, Auto Science, Canada) at 70–90 W for 15 min intervals (allowing the machine to cool for 5 min) until all of the toluene was evaporated. The suspension was filtered first through a 0.7 μm glass filter (Whatman, UK), and then through a 0.22 μm nylon membrane (Whatman, UK). The suspension was concentrated with a rotary evaporator at 45.0 ± 1.0 °C. The concentrated suspension was kept as the nC_{60} stock suspension after being refiltered through a 0.22 μm membrane. The concentration of fullerene stock suspension was detected by UV absorbance at 336 nm (Lyon et al., 2006). Zeta potentials and hydrodynamic particle size of the nC_{60} at different pH values were measured with a ZetaSizer (Malvern Instrument, UK).

Two FA samples were isolated from Amherst peat soil (Amherst, MA) and Beijing soil (Jiufeng National Forest Park, Beijing, China) using the isolation method recommended by the International Humic Substances Society (http://www.humicsubstances.org/soilhafa.html), and the resultant FAs were named as AFA and BFA, respectively. Detailed information regarding Amherst peat soil and Beijing soil has previously been reported (Lin et al., 2011; Yang and Xing, 2009). The C, H, O, N and S contents of the FAs were determined using an elemental analyzer (Elementar, Microcube, Germany) after drying at 105 °C for 36 h. The properties of the FAs are summarized in Table S1. The 150.0 mg FA samples were dissolved in water (about 100 ml), and then the solutions were filtered using a 0.22 μm membrane. The 0.22 μm membranes were dried for 48 h at 45 °C before and after filtering the FA samples. The mass of non-soluble FAs was calculated by the mass difference of the 0.22 μm membranes subsequently, the dissolved FAs were evaluated by reducing the weight of nonsoluble FAs from total FA weight (150.0 mg). The filtered FA solutions were then diluted to 100.0 mg/l as stock solution. All chemicals used were analytical grade unless mentioned otherwise, and all solutions were prepared using ultra-purified water (Milli-Q, Molsheim, France).

2.2. Fluorescence quenching titration experiments

Fluorescence quenching titration experiments of FAs and nC_{60} were evaluated by a modified batch equilibration technique. The nC_{60} suspension of 0–10 ml was added to a series of 15 ml vials with Teflon screw caps. A certain volume of FA stock solutions was then mixed and diluted to 15 ml. Independent kinetic experiments suggested that the quenching of FA by nC_{60} reached equilibrium within 24 h after mixing (Fig. S1). Therefore, all mixture solutions were shaken and stored in the dark for 24 h at their corresponding temperatures to ensure the reactions reached equilibrium. A series of nC_{60} suspensions were prepared and diluted with ultra-purified water as controls.

Within the FA concentration level-dependent experiments, different concentrations of FA (5.0, 10.0, 15.0, and 20.0 mg/l) were titrated by nC_{60} at pH 7.00 ± 0.05 and 18.0 ± 0.5 °C. Within the pH-dependent experiments, 10.0 mg/l FAs were titrated by nC_{60} at pH 3.00 ± 0.05, 5.00 ± 0.05, 7.00 ± 0.05, 9.00 ± 0.05, and 11.00 ± 0.05 respectively, adjusted with HCl and NaOH at 18.0 ± 0.5 °C. For the temperature-dependent experiments, the mixture solutions of 10.0 mg/l FA and nC_{60} were equilibrated for 24 h at 5.0 ± 0.5, 18.0 ± 0.5, 33.0 ± 0.5, 45.0 ± 0.5, and 60.0 ± 0.5 °C using a water bath. The fullerene concentrations kept constant during fluorescence quenching titration in the study evaluated by UV absorbance. Three independent experiments were carried out, and results were reported as their average.

2.3. Fluorescence and UV spectroscopic analyses

Fluorescence spectra were measured with a spectro-fluorometer (LS55, PerkinElmer, USA) with a 150 W xenon arc lamp and a 1 cm quartz cell. Three-dimensional excitation–emission matrixes were recorded at 1200 nm/min and auto-response. The emission fluorescence spectra with excitation wavelength at 325 and 315 nm were collected for AFA and BFA respectively. The emission fluorescence spectra were recorded at 600 nm/min from 200 to 600 nm. The shutter of the monochromator was automatically closed to protect samples from ultraviolet light irradiation during the measurements. The UV–vis spectra were measured with a Shimadzu 2000 spectrometer (Shimadzu Corp. Japan).

The fluorescence excited state lifetimes of AFA with and without nC_{60} were measured using a TemPro Fluorescence Lifetime System (Horiba Jobin Yvon, Edison, NJ). Excitation was provided by the 280 nm pulsed LED 4 operating at a 1 MHz repetition rate. The excitation and emission monochromators were tuned to 280 and 400 nm, respectively during fluorescence lifetime detection. Fluorescence lifetime data were collected and fitted with the DAS6 program of the TemPro Fluorescence Lifetime System.

All the solutions were purged for 15 min using nitrogen to avoid static quenching caused by oxygen before fluorescence spectra detection, as mentioned in previous researches (Pan et al., 2007; Ryan and Weber, 1982).

2.4. Molecular conformation simulation

The three-dimensional structure of FA–nC_{60} complexes was constructed by the molecular modeling (Chemoffice 2004, Cambridge Soft, USA). Chemical structure was geometrically optimized using the MM2 force field and the conjugate-gradient minimizer to an energy change convergence criterion of 0.100 in the Chem3D program. Dynamic properties were also assembled to compute a molecular dynamics trajectory and to explore the conformational space accessible to these complexes. The step interval was fixed at 2.0 fs. The simulated annealing process was computed by applying 20,000 fs molecular dynamics simulation to the target temperature of 300 K.