Molecular characterization of dissolved organic matter in various urban water resources using Orbitrap Fourier transform mass spectrometry

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ABSTRACT

Molecular-level characterization of dissolved organic matter (DOM) in different urban water resources (river water, groundwater, reclaimed water, and rainwater) was performed by Orbitrap Fourier transform mass spectrometry (FT-MS). The mass spectra, which were fingerprints of DOM composition, were clearly different among samples. Based on accurate molecular mass determination, about 300 molecular formulae were identified. Most of the DOM molecules were composed of carbon, hydrogen and oxygen. Molecular formulae in river water consisted of CHO mostly and much less N- and S-containing compounds than other water samples. The percentage of CHO molecules was the highest for reclaimed water. The reclaimed water sample was characterized by a few peaks with strong intensities, which were presumed to be linear alkylbenzene sulfonates, their co-products and their biodegradation products based on their molecular formulae. Some of these compounds were also detected from river water, probably indicating the impact of wastewater treatment plants located upstream of the sampling point. Orbitrap FT-MS analysis is a powerful tool to discriminate DOM composition of urban water resources.

Key words | dissolved organic matter, molecular characterization, Orbitrap Fourier transform mass spectrometry, urban water resources

INTRODUCTION

Climate change can dramatically influence the pattern of precipitation and increase the vulnerability of water resources (Vörösmarty et al. 2000; Charlton & Arnell 2011). In order to adapt for such impacts on the current water system, which basically relies on the restricted water resource such as river water in Tokyo, it is necessary to consider an alternative water resource to establish a resilient system. In urban areas, not only surface water, but also other ubiquitous resources such as groundwater, rainwater, and reclaimed wastewater are available (Furumai 2008). In order to allocate these urban water resources to proper water use, characterization of water quality is imperative. Dissolved organic matter (DOM), which is composite of numerous kinds of organic molecules, is one of the most important components in terms of water use. Some DOM deteriorate the performance of membrane filtration by developing fouling (Zularisam et al. 2006), while some organic substances react with disinfectants and produce toxic disinfection by-products (Kitis et al. 2002). Furthermore, biodegradable fraction of DOM, measured as assimilable organic carbon, can stimulate bacterial regrowth in supply systems of drinking water and reclaimed water (Soonglerdsongpha et al. 2011; Thayanukul et al. 2013). However, only bulk DOM was conventionally analysed by comprehensive indices such as total organic carbon (TOC) and chemical oxygen demand (COD). Although advanced attempts have been applied to characterize DOM based on molecular size distribution, resin fractionation, fluorescence spectra, etc. (Thapa et al. 2002; Nakajima et al. 2003), the molecular-level identification of DOM has not yet been achieved.

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Non-targeted mass spectrometry (MS) analysis for complex samples is realized by ultra-high-resolution mass determination with the advent of Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS). Coupling of electrospray ionization (ESI) with FT-ICR-MS allows us to analyse composite DOM samples and obtain accurate mass of each ionized molecule. Based on very accurate mass, elemental composition or molecular formula for each ion can be precisely determined (Reemtsma 2009). This novel approach was successfully applied to humic substances, which have been operationally defined as a representative component of DOM (Kujawinski et al. 2002; Stenson et al. 2002). In addition, FT-ICR-MS has revealed molecular composition of natural organic matter such as marine DOM (Koch et al. 2005), DOM along a river to ocean transect (Sleighter & Hatcher 2008). Furthermore, the changes in DOM composition along drinking water purification process and wastewater treatment process were evaluated by FT-ICR-MS (Gonsior et al. 2011; Zhang et al. 2012). Combination of FT-ICR-MS with other techniques such as size-exclusion chromatography and excitation–emission matrix fluorescence has also broadened the field of application (Reemtsma et al. 2008; Herzsprung et al. 2012).

Recently, the Orbitrap technology was realized to develop a bench-top electric-field type FT-MS (Makarov 2000). Compared with magnetic-field type FT-ICR-MS, Orbitrap FT-MS is smaller and lower in cost, while the performance is competitive. In this study, we compared the composition of DOM collected from river water, groundwater, rainwater, and reclaimed water with an Orbitrap FT-MS. Molecular-level DOM characterization of these samples is useful to obtain a fingerprint of each water resource.

MATERIALS AND METHODS

Sampling

All glassware used for sampling and following process was immersed in 0.1 M HCl overnight and rinsed thoroughly by Milli-Q water. The glassware and glass fiber filter were baked at 550 °C for 6 hours prior to usage. River water was taken at Shoki-bashi bridge (36.111565° N, 139.197206° E) on Arakawa River in Yorii town, Saitama, in the vicinity of Tokyo, Japan on July 24, 2012. Groundwater and reclaimed water samples were obtained within Arakawa River basin. The groundwater sample was collected at drinking water plant A on August 31, 2012. This plant pumps up deep-well water from 300 m below ground level and distributes water after chlorination and rapid sand filtration. Reclaimed water sample was taken at water reclamation plant B on July 20, 2012. The plant receives water after activated sludge treatment of municipal wastewater. The reclaimed water is produced by biological filtration unit, ozonation and chlorination. Rainwater was taken from a rainwater storage tank which harvests rainwater on a roof of a house in a residential area in Yokohama city, Kanagawa, Japan one day after rainfall on September 7, 2012. The rain on September 6, 2012 was 4.8 mm after 3 days of dry period, and 4.1 mm rain was collected after discarding 0.7 mm initial rainfall. All samples were filtered with a 0.3 μm glass fiber filter (GF-75, Advantec, Japan).

Water quality analysis

Electric conductivity (EC) and pH were measured on site with Model multi 3430 (WTW, Germany). Dissolved organic carbon concentration was determined by TOC-V CSH (Shimadzu, Japan). Ultraviolet absorbance at 254 nm was evaluated using a 5 cm cell by spectrophotometry (U-1500, Hitachi, Japan). The anion concentration (NO₃⁻, NO₂⁻, PO₄³⁻, SO₄²⁻, Cl⁻, Br⁻) was analyzed with an ion chromatograph (IC 761, Metrohm, Switzerland) equipped with a SI-90 4E column (Shodex, Japan). Ammonia was measured spectrophotometrically with HACH DR2800 (Hach, USA).

Solid phase extraction (SPE)

SPE was conducted to concentrate DOM and to eliminate inorganic salt. Samples were acidified to pH 2 with 2 M HCl and passed through a polystyrene-divinylbenzene polymer cartridge (Bond Elut PPL, Agilent Technologies, USA). This cartridge was chosen as it can retain highly polar to nonpolar substances from large volumes of water (Dittmar et al. 2007). Methanol was used to elute the retained organic matter from the SPE cartridge. Different volumes of samples and methanol were used to appropriately concentrate the samples: 2 L samples for groundwater, rainwater and river
water, and 0.5 L for reclaimed water; 20 mL methanol for groundwater, reclaimed water and river water, and 10 mL for rain water. A half of the methanol eluent was used to evaluate organic matter recovery. Dissolved matter was recovered by evaporating methanol to dryness under reduced pressure, and dissolved in Milli-Q water. Recovery ratio was determined based on dissolved organic carbon concentration in the eluents and the original samples.

**ESI-Orbitrap Fourier transform mass spectrometry**

The SPE eluents were analyzed by Orbitrap FT-MS (Exactive, Thermo Scientific, USA) with an ESI source. Samples were injected to the mass spectrometer by flow injection method using an Accela autosampler (Thermo Scientific) at a flow rate of 200 μL/min with methanol. Ionization at injection was in negative ionization mode and source conditions for ESI were as follows: spray voltage, 4.5 kV; sheath gas, 30 arbitrary units (AU); auxiliary gas, 5 AU; sweep gas, 0 AU; capillary temperature, 250 °C; capillary voltage, 25 V; tube lens voltage, 100 V; skimmer voltage, 30 V. Conditions for Orbitrap FT-MS were: resolving power, 100,000; maximum injection time, 250 ms; automatic gain control (AGC) target, 500,000 for full scan analysis (mass range 100–1,000 mass-to-charge ratio, m/z). The instrument was externally calibrated using a calibration mixture provided by the manufacturer at the beginning of the analysis. Additionally, hexadecanoic acid ([M – H]⁻ = 255.23295) in the samples was used for lock mass as internal standards. Blank sample using Milli-Q water was analyzed in the same manner after SPE extraction. All samples were analyzed in triplicate.

**Mass spectra analysis**

The raw mass spectra were processed using a software Sieve version 2.0 (Thermo Scientific) to extract ions in the samples. The triplicated samples were compared with the blank sample, and ions which have statistically significant difference with blank sample and with a signal-to-noise ratio ≥3 were screened. To estimate chemical formula for the ions, we used a software Xcalibur version 2.1 (Thermo Scientific). Carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus and chlorine were included to constitute the formula and the mass tolerance was set at 5 ppm. The nitrogen rule was applied and the relative intensity between 35Cl⁻–containing and 37Cl⁻–containing isotope peaks was used to confirm the number of chlorine atoms in the molecular formula. The following conservative ratios were also applied to validate molecular formulae: N/C < 0.5, S/C < 0.2, P/C < 0.1, (S + P)/C < 0.2, H/C: 0.2–3.1, Cl/C: 0–0.8 and O/C: 0–1.2 (Kind & Fiehn 2007; Sleighter & Hatcher 2008). When the chemical formula cannot be determined within mass error of 5 ppm or by following elemental ratios, those peaks were not used for further analysis. Based on the chemical formula obtained, van Krevelen diagrams (van Krevelen 1950) were plotted with O/C and H/C ratios of the compounds.

**RESULTS AND DISCUSSION**

**Water quality**

Basic water quality data are summarized in Table 1. DOC concentration was above 3 mg/L in the reclaimed water while other samples had approximately 1–2 mg/L. Chloride and nitrate concentration were also much higher in reclaimed water than others. Higher EC in the sample indicated the higher ion concentration in the reclaimed water. Dissolved ion in the water, which originated from the treated wastewater, cannot be basically removed in the treatment processes. In contrast, rainwater had very low EC. It indicated low ion concentration dissolved in the sample. The groundwater sample contained higher ammonia and phosphate concentrations than the other samples, 0.74 and 0.4 mg/L, respectively. Groundwater could be affected by fertilizer applied to the vegetable plantation nearby.

**Solid phase extraction**

Carbon-based recovery by SPE was 49.6, 55.2, 77.7 and 48.5% for river water, groundwater, rainwater and reclaimed water, respectively. The recovery was around 50% except for the rainwater sample. The highest recovery for the rainwater indicated that the sample contained more compounds which can be recovered by the SPE. Such compounds could be hydrophobic DOM molecules.
Mass spectra pattern

The patterns of mass spectra of each of the water samples obtained by Orbitrap FT-MS are shown in Figure 1. The mass spectra were clearly different among samples. This indicates that the DOM composition in each urban water resource is different. Weighted averages of neutral mass were 312.4, 319.9, 230.7 and 248.6, for river water, groundwater, rainwater and reclaimed water, respectively. The rainwater and reclaimed water samples had smaller molecules compared with the other samples. While this analysis targeted m/z ranged from 100 to 1,000, more and stronger peaks were found in the smaller molecules which had m/z in the range of 100 to 400. Based on molecular

<table>
<thead>
<tr>
<th>Samples</th>
<th>pH (-)</th>
<th>EC (mS/cm)</th>
<th>UV&lt;sub&gt;254&lt;/sub&gt; (5 cm⁻¹)</th>
<th>DOC (mg L⁻¹)</th>
<th>Cl⁻ (mg L⁻¹)</th>
<th>NH₄-N (mg L⁻¹)</th>
<th>NO₂-N (mg L⁻¹)</th>
<th>NO₃-N (mg L⁻¹)</th>
<th>PO₄-P (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>River water</td>
<td>8.67</td>
<td>139</td>
<td>0.11</td>
<td>0.84</td>
<td>2.2</td>
<td>0.03</td>
<td>&lt; 0.03</td>
<td>0.6</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td>Groundwater</td>
<td>8.05</td>
<td>176</td>
<td>0.39</td>
<td>1.55</td>
<td>2.5</td>
<td>0.74</td>
<td>&lt; 0.03</td>
<td>&lt; 0.02</td>
<td>0.4</td>
</tr>
<tr>
<td>Rainwater</td>
<td>6.86</td>
<td>25</td>
<td>0.12</td>
<td>1.72</td>
<td>0.3</td>
<td>0.10</td>
<td>&lt; 0.03</td>
<td>0.5</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td>Reclaimed water</td>
<td>7.61</td>
<td>403</td>
<td>0.23</td>
<td>3.33</td>
<td>40.7</td>
<td>0.08</td>
<td>&lt; 0.03</td>
<td>10.4</td>
<td>&lt; 0.03</td>
</tr>
</tbody>
</table>

Figure 1 | Mass spectra of Orbitrap FT-MS analysis: (a) river water, (b) groundwater, (c) rainwater, and (d) reclaimed water.
mass and according to the conditions indicated at materials and methods, 293, 300, 300, and 300 molecular formulae were identified for river water, groundwater, rainwater, and reclaimed water, respectively.

The mass spectrum of reclaimed water contained a few peaks with strong intensities. Some of the assigned molecular formulae of these peaks contained sulfur, and the formulas were the same as linear alkylbenzene sulfonates (LAS) and their biodegradation products sulfophenyl carboxylates, SPC (for example, C9H12O3Sa and C13H18O5S, respectively). In addition, molecules which were likely to be dialkyl tetralin-sulfonates, major co-products of LAS and its intermediate were also observed (for example, C13H18O3S and C14H18O5S, respectively). River water also contained some of the LAS-related compounds. As there are three wastewater treatment plants located upstream of the sampling point of the river water, they were probably derived from the effluent from wastewater treatment plants. These LAS-related compounds have been reported well in the secondary effluent (Corcia et al. 2013) or river water (Trehy et al. 1996). The results obtained in this study imply the importance of the compounds among the vast number of organic matters in reclaimed water and river water.

Van Krevelen diagram

The numbers and composition of all assigned molecular formulae are summarized in Table 2. Most formulae were composed of carbon, hydrogen and oxygen. River water contained more than 97% of CHO compounds and much fewer other compounds. The other samples were composed of more numbers of CHON and CHOS compounds. Most CHOS compounds were identified in the reclaimed water. Most of them were likely to be surfactants or their derivatives. Nitrogen- and sulphur-containing compounds were also found in the groundwater. Longnecker & Kujawinski (2011) also reported N- and S-containing compounds in groundwater samples and they estimated their origins to be from rainwater and surface water. The H/C ratio was smallest in groundwater and second smallest in river water. The smaller H/C ratio might indicate dominance of aromatic compounds which derive from the polymeric aromatic compounds such as humic substances. The averages of O/C ratio were not different among the samples. Elemental ratios of O/C and H/C were used to plot van Krevelen diagrams (Figure 2), which demonstrate DOM composition in the samples. The plots for the river water were mainly located in small area, 0.6 < H/C < 1.5 and 0.2 < O/C < 0.6, where lignin-like molecules are mostly found (Sleighter & Hatcher 2007). DOM in the river water mainly consisted of lignin-like molecules. Other samples have more scattered plots, which indicate that they have more diverse kinds of compounds.

CHON compounds were typically found in the zone H/C < 1 and O/C < 0.4 in all samples. CHON compounds had average H/C of 1.8 in rainwater directly analysed by FT-ICR-MS (Altiere et al. 2009). The difference may be stemmed from the different treatment of the samples before analysis and/or from the different location.

CONCLUSION

Composition of DOM in different urban water resources, namely river water, groundwater, reclaimed water and rainwater, was evaluated by Orbitrap FT-MS. Different mass spectra indicated different fingerprints of DOM composition. River water contained mostly CHO compounds. Reclaimed water consisted of S-containing compounds, which were presumed to be linear alkylbenzene sulfonates, their co-products and their biodegradation products. Some of them

<table>
<thead>
<tr>
<th>Samples</th>
<th>CHO</th>
<th>CHON</th>
<th>CHOS</th>
<th>CHONS</th>
<th>Others</th>
<th>H/C ratio</th>
<th>O/C ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>River water</td>
<td>283</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>1.15 ± 0.24</td>
<td>0.46 ± 0.13</td>
</tr>
<tr>
<td>Groundwater</td>
<td>196</td>
<td>70</td>
<td>20</td>
<td>1</td>
<td>13</td>
<td>0.99 ± 0.38</td>
<td>0.45 ± 0.26</td>
</tr>
<tr>
<td>Rainwater</td>
<td>205</td>
<td>59</td>
<td>25</td>
<td>6</td>
<td>5</td>
<td>1.22 ± 0.41</td>
<td>0.46 ± 0.19</td>
</tr>
<tr>
<td>Reclaimed water</td>
<td>164</td>
<td>40</td>
<td>72</td>
<td>5</td>
<td>19</td>
<td>1.26 ± 0.34</td>
<td>0.42 ± 0.22</td>
</tr>
</tbody>
</table>
were also found in the river water, which indicated the impact of wastewater treatment plants located upstream. Orbitrap FT-MS analysis has a great potential to distinguish DOM composition of urban water resources. It can be further applied to specify the compounds of importance for water use, such as precursors of disinfection by-products and substrates for microbial regrowth. Moreover, the analysis can be used to detect unknown compounds at water quality accident and facilitate the identification of the causative compounds.

ACKNOWLEDGEMENTS

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