

Article

Chemical properties of aquatic fulvic acids isolated from Lake Biwa, a clear water system in Japan

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Abstract

Using a large-scale system for the separation and concentration of aquatic humic substances, a fulvic acid (FA) sample was isolated from the surface water in Lake Biwa (156 m³), in which this had been known as a clear water system in Japan, and was sampled during the autumn of 2001. We obtained a powdered sample (18 g) of the Lake Biwa fulvic acid (LBFA) and compared its chemical properties with those of aquatic and soil FAs in previous reports. Liquid-state ¹H and ¹³C nuclear magnetic resonance (NMR) spectroscopy revealed that the LBFA was higher aliphatic and lower aromatic properties than FAs from colored waters and soils. These results in the elemental analysis also supported such characteristics, determined from the NMR spectra. The high-performance size-exclusion chromatography (HPSEC) revealed that the LBFA had at least several major components with different molecular weight distributions. The weight-average molecular weight of the LBFA was estimated to be 884 Da (relative to the standard samples of polystyrene sulfonate sodium). The three-dimensional fluorescence spectrum indicated a maximum peak corresponding to the humic-like fluorophores, but no other distinct peak. These results led to a conclusion that LBFA is mainly derived from degraded and modified materials of microbial residues and microbial metabolites in the Lake Biwa.

Introduction

Aquatic humic substances (AHSs) are complex, heterogeneous organic macromolecules that are chemically or biologically synthesized from biogenic substances (e.g. plankton, microorganisms, etc.) or their degradation products (Aiken et al., 1985), occur in all types of natural waters (rivers, lakes, seas, or groundwater) in the world (Stevenson, 1994), and forms complexes with organic pollutants, heavy metals, radionuclides, and mineral nutrients (Aiken et al., 1985; Steinberg, 2003; Tipping, 2002). Therefore, it is important to examine the chemical properties of AHSs and their interactions with other organic or

mineral compounds in aquatic environments.

The inherent chemical properties of AHSs are determined on the basis of both their place of origin and the genetic environment (Malcolm, 1990; Thomsen et al., 2002). Many researchers have used commercial humic samples from chemical companies such as Sigma-Aldrich Co. (St. Louise, USA), Wako Pure Chemical Industries Ltd. (Osaka, Japan), and Nakarai Tesque Inc. (Kyoto, Japan). However, these commercial products are not considered to be appropriate for the use as analogues of true soil and aquatic humic substances (Malcolm and MacCarthy, 1986). The International Humic Substances Society (IHSS) provides several standard and reference AHS

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samples obtained from natural waters: Suwannee River FA; Nordic Lake FA; Pony Lake FA (IHSS home page; <http://www.ihss.gatech.edu/>). However, chemical properties of the IHSS samples are considerably different from those of AHSs derived from clear water systems that are distributed widely in the temperate zones. For example, Suwannee River and Nordic Lake FAs were prepared from a river and a lake existing in a peaty area, respectively, and Pony Lake FA was isolated from fresh pond water with a high dissolved organic C (DOC) concentration (up to 100 mg C L^{-1}) and high salinity (McKnight et al., 1994). Hence, if one would design to elucidate the nature and reactivity of AHS in clear water, isolating AHS samples from appropriate clear water systems should be required. However, technical difficulties lay in AHS mass isolation due to its low concentrations in clear water. As a result, limited information is available on the properties of AHS in clear water systems.

To overcome this problem, we developed a large-scale preparative system from the separation and concentration of AHS from clear water systems. By using this apparatus, we attempted to obtain large amounts of an AHS sample from Lake Biwa in Japan for use as standard samples from clear water system. Lake Biwa is considered to be the best location for collecting AHS samples from typical clear water, because it has the largest water surface area and impoundment in Japan. Since the quality of the humic acid (HA) sample obtained from Lake Biwa by normal treatments was undesirable because of the high ash content, we focused on the Lake Biwa fulvic acid (LBFA) only. The purpose of this study was to elucidate the chemical characteristics of the LBFA by means of elemental analysis, liquid-state nuclear magnetic resonance (NMR) spectroscopy, high-performance size-exclusion chromatography (HPSEC), and three-dimensional (3D) fluorescence spectrophotometry. To reveal the differences and similarities between the samples, we also compared the chemical characteristics of LBFA with those of FAs obtained from other environmental sources such as soils, waters, and so on.

Materials and methods

Samples

The surface water was collected in north basin of Lake Biwa–Kitakomatsu, Otsu, Shiga Japan, in Au-

turn 2001 (between 17 October and 9 November) (Fig. 1). The collection point was 50 m away from the shore and 50 cm in depth.

Lake Biwa is the largest lake in Japan, located at Shiga prefecture, and originated in the Pliocene about four million years ago. The area is 670.25 km^2 , and the impoundment is 275 km^3 . The lake has 3174 km^2 of the watershed. Eighty seven percent of the soil around the watershed is classified as brown forest soil. Mean annual precipitation in the area is 1584 mm .

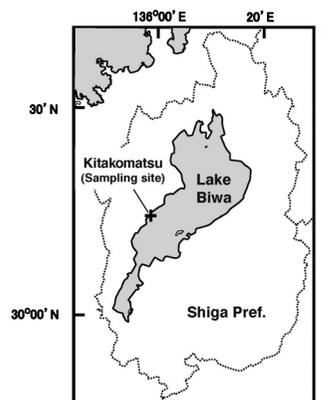


Fig. 1. Sampling site of LBFA –Kitakomatsu, Otsu in Shiga, Japan.

Isolation of LBFA

Using a system for the separation and concentration of AHS (Fig. 2) that was settled at the lakeshore near of the water collection point, the AHS in Lake Biwa was isolated. Approximately 10 m^3 of water per day was continuously filtrated ($<0.45 \mu\text{m}$), adjusted to pH 2 using HCl, and passed through 40 kg of DAX-8 resin (Supelite DAX-8, Sigma-Aldrich Co., St. Louis, USA), in which the 156 m^3 of water were totally applied to the system on site; subsequently, the DAX-8 resin adsorbing the AHS was carried to the laboratory under cool temperature (5°C). Treatment procedures for the DAX-8 resin adsorbing the

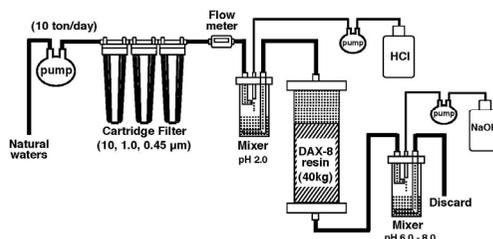


Fig. 2. A separation and concentration system of humic substances in clear water.

AHS in the laboratory were based on the IHSS method (Thurman and Malcolm, 1981). The AHS was eluted from the resin using 0.1M NaOH aqueous and HA and FA were then separated by adjusting the solution to pH 1.5. The FA (supernatant) was converted to the hydrogen form by passing through an H⁺-type cation-exchanger (Amberlite IR120-B, Organo Co., Tokyo, Japan). Finally, powdered FA sample was obtained by freeze-drying. First, we checked the elemental compositions, and found to be high ash content (more than 10%) in the first isolated FA. This may affect the NMR analysis and 3D-fluorescence spectroscopy. Therefore, the FA should be re-purified by HCl-HF treatment twice (Swift, 1996). The ash content in the LBFA was reduced up to 2.1%. Nagao et al. (2007) also describe the detailed procedures for isolation and purification of the LBFA.

Elemental Analysis

The elemental analysis was done by a CHNS/O analyzer (2400II, ParkinElmer Japan Co., Yokohama, Japan), using 2 mg of the dry sample. Ash content of the LBFA was determined after combustion of 10 mg of the dry sample at 550°C in a muffle furnace for 4 hours.

Liquid-state NMR Spectroscopic Analysis

The experiments were performed with a Bruker AVANCE 500 spectrometer (Bruker GmbH, Karlsruhe, Germany) operating at 500.13 MHz for ¹H and at 125.77 MHz for ¹³C. Sample tubes of 5 mm in diameter were used. Solution of the LBFA was prepared by dissolving 50 mg of sample in a mixture of 0.02 mL of 5 M NaOD (99.99%, Sigma-Aldrich Co., St. Louise, USA) and 0.4 mL of D₂O (99.99%, Sigma-Aldrich Co., St. Louise, USA) solution. For the chemical shifts, sodium 3-trimethylsilylpropionate-2, 2, 3, 3, D₄ (TMSP; Euriso-top, Saint Aubin, France) was used as a reference material (0 ppm). ¹H signals were obtained by the homo-gate decoupling technique under following conditions: pulse width 90°; acquisition time 5.4 s; pulse delay 4.8 s. The HOD proton (4.8 ppm) of water impurities was irradiated, and 8 scans were accumulated. ¹³C signals were collected basing on proton decoupling by the inverse gated decoupling technique as following conditions: pulse width 45°; acquisition time 0.2 s. A total repetition time of 2.5 s was applied to permit relaxation of all the spins, and 10000 scans were accumulated. To improve the signal-to-noise ratio, a line broadening

of 20 Hz was employed.

Chemical shift assignments were referred to Wershaw (1985) and Kawahigashi et al. (1996) for ¹H-NMR spectroscopy, and Wilson (1980) and Fujitake and Kawahigashi (1999) for ¹³C-NMR spectroscopy. Aromaticity was calculated by expressing the aryl and *O*-aryl C (110-165 ppm) as a percentage of the alkyl, *O*-alkyl, aryl and *O*-aryl C (5-165 ppm) (Hatcher et al., 1981; Watanabe and Fujitake, 2008).

HPSEC Analysis

The HPSEC system was consisting with a Waters 600E pump, a Waters 717 plus autosampler, a Waters 2487 UV-visible detector, and a Waters 2410 refractive index detector (Waters Co., Milford, USA). A column (Shodex SB803 HQ, 8.0×300 mm (φ×L), exclusion limit of 100,000 Da, Showa Denko Co., Tokyo, Japan) and a guard column (Shodex OHpak SB-G, 6.0×50 mm (φ×L), Showa Denko Co., Tokyo, Japan) were used. The mobile phase consisted of a 0.01 M phosphate buffer at pH 7 (15%) and acetonitrile (25%). A 30 μL aliquot of the sample solution (ca. 0.2 mg L⁻¹) was injected into the HPSEC system. Molecular weight of LBFA was calculated by a GPC analysis software (Millennium 32-J, Nihon Waters Co., Tokyo, Japan) using standard samples of polystyrene sulfonate sodium salt (PSSNa). Other detailed procedures are described in a previous report (Asakawa et al., 2008).

Fluorescence Spectrophotometry

A Hitachi F-4500 fluorescence spectrometer (Hitachi High-Technologies Co., Tokyo, Japan) with a 150-W ozone-free xenon lamp was used to obtain three-dimensional excitation-emission matrix (3D-EEM) contour plot of aqueous LBFA according to the method by Nagao et al. (1997). The 10 mg L⁻¹ of LBFA aqueous in 0.01 M NaClO₄ at pH 8.0 was placed to a quartz cell (10 mm x 10 mm) and then set to the spectrometer. The spectrum was collected at excitation wavelength (Ex.) from 200 to 500 nm and emission wavelength (Em.) from 200 to 600 nm at 5 nm intervals, and voltage of photomultiplier voltage was set at 400 V. Relative fluorescence intensity (RFI) of the samples was expressed, in terms of quinine standard unit (QSU). Ten QSU corresponds to the fluorescence intensity of quinine sulfate (10 μL⁻¹ in 0.05 M sulfuric acid aqueous) at an excitation wavelength of 350 nm and an emission wavelength of 455 nm.

Results and Discussion

Amounts of AHS

The average DOC concentration at the sampling sites in Lake Biwa was 1.3 mg C L^{-1} during the study period (water information system maintained by the Ministry of Land, Infrastructure and Transport, Japan; <http://www1.river.go.jp>). This value is lower than the DOC concentrations of colored river waters ($5\text{--}50 \text{ mg C L}^{-1}$) (Malcolm, 1985) or lake waters (ca. 15.2 mg C L^{-1}) (Steinberg and Muenster, 1985) in the United States. It is also considerably lower than the DOC concentrations in Suwannee River (ca. $25\text{--}75 \text{ mg C L}^{-1}$) (Averett et al., 1994), Pony Lake (up to 100 mg C L^{-1}) (McKnight et al., 1994), and Helle-rudmyra Tarn (ca. $10\text{--}25 \text{ mg C L}^{-1}$) (Averett et al., 1994), which are the sampling sites from where the IHSS standard and reference samples, namely Suwannee River FA, Nordic Lake FA, and Pony Lake FA, respectively were procured (IHSS home page; <http://www.ihss.gatech.edu/>). On the other hand, the DOC concentrations in fresh river waters ($1.5\text{--}10 \text{ mg C L}^{-1}$) (Malcolm, 1985) or groundwater ($0.2\text{--}13 \text{ mg C L}^{-1}$) (Thurman, 1985) are similar to that in Lake Biwa. Therefore, the Lake Biwa water sample used in this study was regarded as a typical clear water sample in terms of DOC level.

Using our novel large-scale preparative isolation system, we obtained 18.0 g of LBFA and 9.6 g of HA

(poorly cleanup fraction) from 156 m^3 of water from Lake Biwa. The LBFA concentration, which was estimated from its yield, was 0.12 mg L^{-1} ($18.0 \text{ g} / 156000 \text{ L}$). This means that the percent C of the LBFA can be estimated to be ca. 5.1% of DOC in the sample water ($0.12 \text{ mg L}^{-1} \times 0.56 / 1.3 \text{ mg C L}^{-1}$; where 0.56 was C ratio by elemental data in Table 1). This value was considerably lower than the average percentages of FAs in fresh river waters (45%) (Malcolm, 1985) or in Lake Biwa water (15–41%) (Sugiyama et al., 2005). This suggests that there may be scope for improving the preparative isolation system to increase the FA recovery rate.

Elemental Composition

Elemental composition of LBFA and other FAs in the literatures are summarized in Table 1. The elemental composition of LBFA was similar to that of Lake Fryxell and Lake Hoare FAs, which were isolated from Antarctic clear lake waters (McKnight et al., 1994). However, C and N contents in the LBFA were different from those of Pony Lake FA, which was from another Antarctic clear lake water and was one of the IHSS reference samples. The C, H and N contents in the LBFA were higher than those in the colored waters (Suwannee River and Nordic Lake FAs) and those in clear water (Ogeechee Stream FA). According to McKnight et al. (1991) and Mao et al. (2007), the high N contents in the three of Antarctic

Table 1. Elemental Composition (Weight %, ash and water content free) and H/C and O/C ratio of LBFA and those of other FAs from literatures listed in the table.

Sample	C%	H%	N%	O%	H/C	O/C	Reference
LBFA	56.1	6.06	2.31	35.5	1.30	0.47	
Lake Fryxell FA	55.0	5.50	3.10	34.9	1.20	0.48	McKnight et al. (1994)
Lake Hoare FA	54.9	5.50	2.90	35.7	1.20	0.49	McKnight et al. (1994)
Pony Lake FA ¹	52.5	5.39	6.51	31.4	1.23	0.45	http://www.ihss.gatech.edu/
Nordic Lake FA ²	52.3	3.98	0.68	45.1	0.91	0.65	http://www.ihss.gatech.edu/
Suwannee River FA ³	52.4	4.31	0.72	42.2	0.99	0.60	http://www.ihss.gatech.edu/
Ogeechee Stream FA	54.6	4.97	0.87	38.2	1.09	0.52	Malcolm (1985)
fresh water FA (n=63)*	46.7	4.20	2.30	45.9	1.10	0.75	Rice and MacCarthy (1991)
groundwater FA (n=3)*	60.1	5.70	0.90	31.6	1.13	0.40	Thurman (1985)
marine FA (n=12)*	45.0	5.90	4.10	45.1	1.56	0.77	Rice and MacCarthy (1991)
lake-sediments FA (n=5)*	45.0	5.12	7.63	42.3	1.34	0.72	Ishiwatari (1985)
soil FA (n=127)*	45.3	5.00	2.60	46.2	1.35	0.78	Rice and MacCarthy (1991)

¹ IHSS reference sample (1R109F). ² IHSS reference sample (1R105F). ³ IHSS standard sample (1S101F). * average values of samples.

clear water lake FAs may be explained by the absence of lignaceous precursors in the diagenetic formation of dissolved FAs; lignaceous precursors containing no N would be diluents of the N content of dissolved FAs formed in the other aquatic environments. Therefore, LBFA may be less influenced by lignin components derived from terrestrial plants.

The average elemental contents of FAs, isolated from various sources, are also listed in Table 1. However, these differences were not sufficiently prominent. In fact, the C content of LBFA was substantially different from the average C contents of fresh water FAs, lake- sediment FAs, marine FAs and soil FAs, respectively (Ishiwatari, 1985; Rice and MacCarthy, 1991), but similar to the average N contents of fresh water FAs and soil FAs (Rice and MacCarthy, 1991). The diagram of H/C vs. O/C ratio, referred as the van Krevelen diagrams (van Krevelen 1961), has been often employed by various workers to illustrate compositional differences among HAs and FAs from various origins. The LBFA plotted in the diagram (Fig. 3) occurs in the upper area of soil HAs and the left outside of soil FAs' area (Yonebayashi and Hattori, 1988), indicating high aliphatic properties and a low degree of humification (Kuwatsuka et al., 1978). Although the three of Antarctic clear lake water FA lie adjacent to LBFA, the LBFA separated from other FAs and commercial humic substances. Although these results are not convincing enough to concretely define the differences or similarities between LBFA and other humic samples, they provide supportive evidence for the interpretation of the NMR data, as described in the following section.

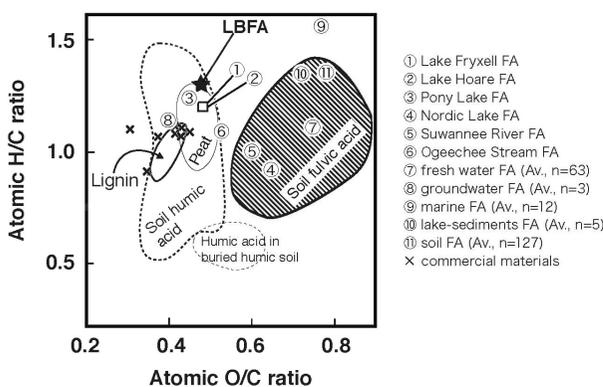


Fig. 3. Diagram of H/C versus O/C ratios of LBFA. Surrounded areas are illustrated by referring to Yonebayashi and Hattori (1988). "×" in Fig.3 indicates commercial samples from Malcolm and MacCarthy (1986). Other symbols were plotted from data in Table 1.

NMR Spectra

The ^1H -NMR spectrum of LBFA (Fig. 4) exhibited large sharp signals originating from protons of terminal methyl groups attached to saturated aliphatic C in the range from 0.0–0.9 ppm. These signals are commonly observed in the ^1H -NMR spectra of FAs isolated from aquatic environments (e.g., Suwannee River FA) (Thorn et al., 1989). The signals of protons on C attached to oxygen-sugars, olefins, and methoxyl groups (3.0–4.3 ppm) were smaller in the LBFA spectrum than in the spectra of soil FAs (Thorn et al., 1989) or HAs (Kawahigashi et al., 1996; Fujitake et al., 2003; Fujitake, 2007). Moreover, the signals of protons attached to C of heteroaromatic and aromatic rings, and carbonyl groups-bonded to electronegative groups (6.0–9.0 ppm) were minimally detected in the LBFA spectrum, unlike in the spectra of typical aquatic or soil FAs (Thorn et al., 1989; Malcolm, 1990; Fujitake, 2007).

The quantitative ^{13}C -NMR spectrum of LBFA (Fig. 5) exhibited four major broad peaks in the regions of 10–66 ppm, assigned to alkyl and partly to *O*-alkyl C; 66–100 ppm, assigned to *O*-alkyl C; 110–160 ppm, assigned to aryl and *O*-aryl C; and 160–190 ppm, assigned to carboxylic C. The broad peaks that were assigned to alkyl and *O*-alkyl C (10–66 ppm) were considerably higher than those assigned to aryl and

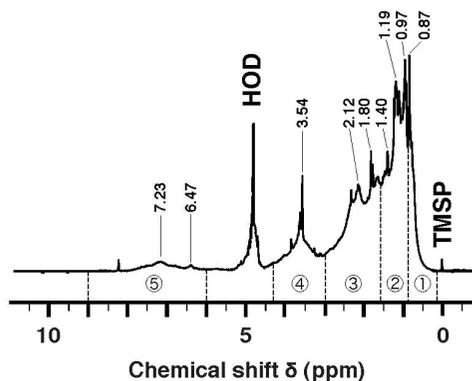


Fig. 4. ^1H NMR spectrum of LBFA

① 0–0.9 ppm (protons on the terminal methyl groups attached to saturated aliphatic protons); ② 0.9–1.6 ppm (protons on methylene β attached to olefins or aromatic rings); ③ 1.6–3.0 ppm (protons on methyl and methylene α attached to aromatic carbons, carbonyl groups, ester groups, and olefins); ④ 3.0–4.3 ppm (protons on the carbon attached to oxygen-sugars, olefins, and methoxyl groups); ⑤ 6.0–9.0 ppm (protons attached to the carbon of heteroaromatic and aromatic rings and to the carbonyl groups bonded to electronegative groups)

O-aryl C (110–160 ppm) or carboxylic C (160–190 ppm) in the LBFA spectrum. This indicates that LBFA has aliphatic rich properties. These results are similar to those obtained for other aquatic FAs (e.g., Suwannee River and Pony Lake FAs) in the previous reports (Thorn et al., 1989; McKnight et al., 1994).

In addition, the ^{13}C -NMR spectrum of LBFA exhibited many well-resolved and sharp peaks that superimposed on the major broad peaks. These features have never been observed in the spectra of other aquatic or soil FAs. The most intense peaks in the LBFA spectrum were apparent at approximately 29 ppm and 39 ppm, and the other peaks were observed at approximately 63, 75, 120, 142, and 186 ppm. The presence of well-resolved peaks in the spectrum suggests that some other simple chemical compounds may incorporate into and/or coexist with the LBFA molecule.

The composition of C species in LBFA, as estimated on the basis of the ^{13}C -NMR spectrum, is presented in Table 2. The proportion of alkyl- + *O*-alkyl-C species (5–110 ppm) for the LBFA (62.9%) was similar to those for Pony Lake and Lake Fryxell FAs (63–70%) (McKnight et al., 1994), but was considerably higher than those for Suwannee River FA (IHSS home page: <http://www.ihss.gatech.edu/>), Inogashira and Dando FAs (42–49%) (Fujitake, 2007). Moreover, the aromaticity for the LBFA (0.21) was lower than those for Suwannee River,

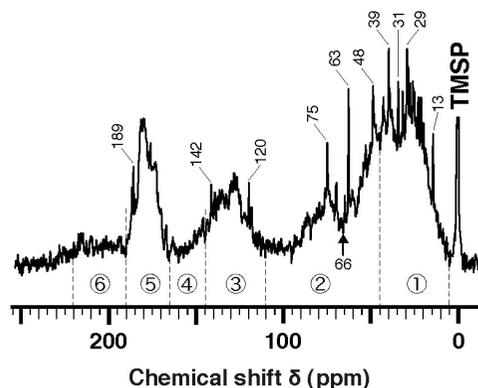


Fig. 5. ^{13}C NMR spectrum of LBFA

① 5–45 ppm (alkyl C); ② 45–110 ppm (*O*-alkyl C); ③ 110–145 ppm, aryl C; ④ 145–165 ppm (*O*-aryl C); ⑤ 165 to 190 ppm (carboxylic C); ⑥ 190–220 ppm (carbonyl C)

Inogashira, and Dando FAs (0.33–0.42). The Pony Lake and Lake Fryxell FAs were considered to have originated from algae and other plankton, because these lake waters in the Antarctic region are rich in plankton and the lake surroundings are not covered with a higher plant. The similarities between the LBFA and Pony Lake and Lake Fryxell FAs suggest less conspicuous contribution of lignin to LBFA.

Molecular Weight Distribution

The LBFA schromatogram obtained by HPSEC is

Table 2. C species composition in LBFA estimated on the basis of the ^{13}C -NMR spectrum and those in other FAs from literatures listed in the table.

Sample	C species % (δ ppm)						Aromaticity	Reference
	alkyl-C (5-45)	<i>O</i> -alkyl-C (45-110)	aryl-C (110-145)	<i>O</i> -aryl-C (145-165)	COO-C (165-190)	CO-C (190-220)		
LBFA	39.4	23.5	13.9	3.2	14.3	5.6	0.21	
Pony Lake FA ¹	62.9		17.1		17	1.2	0.15	http://www.ihss.gatech.edu/
Lake Fryxell FA	63.1		11.9		20.7	4.4	0.16	McKnight et al. (1994)
Nordic Lake FA ²	37		31		24	10	0.46	Thorn et al., (1989)
Suwannee River FA ³	49		24		20	7	0.33	Thorn et al., (1989)
Inogashira FA ⁴	43.8		30.0		22.9	3.3	0.41	Fujitake (2007)
Dando FA ⁴	41.9		30.2		24.0	3.9	0.42	Fujitake (2007)

¹ IHSS reference sample (1R109F). ² IHSS reference sample (1R105F). ³ IHSS standard sample (1S101F). ⁴ JHSS (Japanese humic substances society) standard samples. * Aromaticity = (% of 110–165 ppm) / (% of 5–165 ppm)

shown in Fig. 5. Two apparent peaks were observed at molecular weights of 995 and 785 Da, relative to the standard samples of PSSNa at pH 7 at 260 nm. In addition, two shoulder peaks were observed at approximately 530 and 470 Da. These data suggest that the LBFA contained at least several major components.

The weight- and number-average molecular weights of the sample were determined to be 884 and 604 Da, respectively. The former value was considerably smaller than those for various soil HAs (3160 to 26400 Da), which were measured under same conditions in this study (Asakawa et al., 2008), and than that for Suwannee River FA (2310 Da) (Chin et al., 1994). HPSEC is a popular analytical technique used for determining the molecular weights of humic substances (Chin et al., 1994; Piccolo et al., 2002; Hoque et al., 2003). However, the optimization of the analytical conditions for HPSEC analysis of humic substances is difficult, and standard method for investigations involving various types of humic materials (e.g., soil and aqueous FAs and HAs obtained from coal and manure) has not been established thus far. Even when same sample is applied, the use of different methods or conditions leads to varying molecular weights. Therefore, it was difficult to compare the molecular weights determined in this study with those for published data previously for other FAs.

Fluorescence Spectra

A 3D-EEM contour plot of LBFA is shown in Fig. 6. The plot exhibited only one maximum peak (fluorescence at Ex. 300 nm/ Em. 425 nm) and no other distinct peaks. The maximum peak was distributed in the optical space of “humic-like fluorophores” (Coble, 1996; Hudson et al., 2007). The humic-like fluorophores are commonly observed in freshwater DOM or aqueous FA (Senesi, 1990; Mobed et al., 1996; Nagao et al., 2003; Cory and McKnight, 2005; Mladenov et al., 2007), and are likely originated from the breakdown of organic material in water, riparian zones and other soils (Katsuyama and Nobuhito, 2002).

Although another fluorophores in the range of Ex. 265-290 nm/ Em. 300-350 nm, so-called “protein-like (specifically tryptophan- or tyrosine-like) fluorophores”, are frequently observed in freshwater DOM or aqueous FA (Wu & Tanoue 2001; Cory and McKnight, 2005), no peaks in the optical space ap-

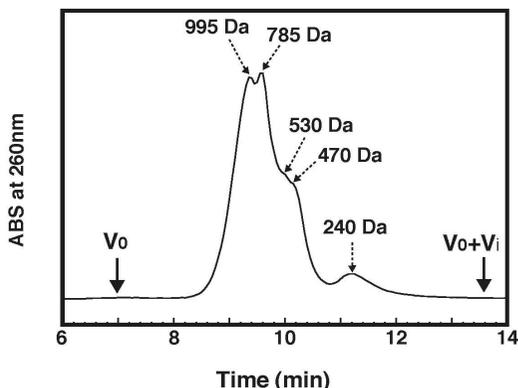


Fig. 6. HPSEC chromatogram of LBFA.

V_0 , void volume; $V_0 + V_i$, total effective column volume

peared in the LBFA. Such protein-like peaks have been found to be linked to bacterial activity, to sewage treatment process efficiency and therefore to organic matter bioavailability (Hudson et al., 2007). The spectrum for LBFA without the protein-like peaks suggests less contribution of fresh or living matter and anthropogenic inputs. This was supported by the lower weight-average molecular weight of LBFA, because fresh, namely no degraded, proteins and polysaccharides had relatively high molecular weights.

Conclusions

We have studied the chemical characteristics of a clear lake water FA in the temperate zones including Japan. The elemental compositions for the LBFA

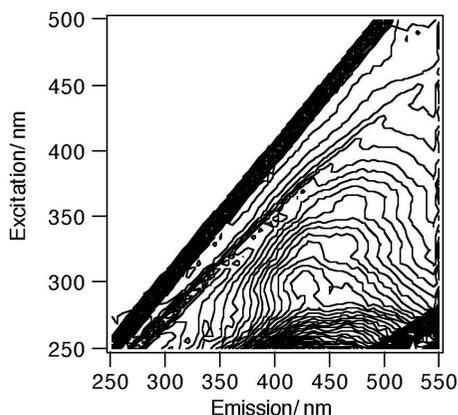


Fig. 7. 3D-EEM contour plot of LBFA.

The concentration of LBFA was 10 mg L^{-1} in 0.01 M NaClO_4 at pH 8. The intervals of the contour are 2 QSU.

were different from those for commercial, aquatic and soil humic samples, except for those for FAs from Antarctic clear lake waters. The elemental composition and ^1H and ^{13}C NMR spectra showed the aliphatic nature of the LBFA. Higher N contents and proportion of C species for the LBFA were analogous to those for three FAs from Antarctic clear lake waters. This suggests that the LBFA is less influenced by lignin derived from terrestrial plants. The HPSEC chromatogram revealed that the LBFA had at least several major components with different molecular distributions. The fact that no protein-like peaks observed in the 3D-EEM contour plot suggested less contribution of fresh or living matter and anthropogenic inputs to LBFA. This was supported by the lower weight-average molecular weight of LBFA. In conclusion, the LBFA is mainly derived from degraded and modified materials of microbial residues and metabolites in Lake Biwa.

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