

Determination of Dissolved and Particulate Thiols in Lake Biwa by Optimized HPLC-Fluorescence Method to Observe Their Distribution

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Thiols (-SH functional group compounds) are important antioxidants and metal-complexing agents that have the ability to alter the biogeochemistry of many chalcophile elements by reducing the free-metal concentrations in phytoplankton, plants, and fungi exposed to high concentrations of metals. Fulvic acids (FAs) is mixture of ligands with aromatic masses ranging from a few hundred to thousands of atomic units and has a wide variety of multiple aromatic and aliphatic functional groups that have the affinity as metal binding sites. Thiols detection remains challenging for their extreme low concentration in both their reduced (R-S) and oxidized (R-S-S-R) forms in aquatic environment.

A modified biochemical method that was developed in our laboratory applied to determine the levels of dissolved and particulate thiols in vertical profile of Lake Biwa water and extracted Lake Biwa fulvic acids (LBFAs) obtained at various depths. This method involved the reduction of thiols by tris (2-carboxyethyl) phosphine and the derivatization of thiols into fluorescent compounds by 7-fluorobenzo- furazan-4-sulfonic acid ammonium salt (SBD-F). These procedures were followed by solid-phase extraction with solid phase extraction, and chromatographic separation by ODS column and fluorescence detection. In summer, dissolved cysteine (Cys) (2.0-6.0 nM), glutathione (GSH) (2.8-5.1 nM), and N-acetyl-L-cysteine (NAC) (1.6-4.2 nM) were detected throughout the water column but were broadly consistent at depths of 5-20 m (Fig. 1). In winter, dissolved Cys (2.7-4.5 nM), GSH (1.1-7.7 nM) and NAC (0.9-1.7 nM) were found in Lake Biwa. In contrast, abundant levels of particulate Cys ($1.3-3.5 \times 10^2$ nM) and GSH ($1.6-3.1 \times 10^2$ nM) were detected down to depths of 15 m in summer. Although, particulate Cys ($1.03-2.2 \times 10^2$ nM) and GSH ($0.8-1.4 \times 10^2$ nM) were measured throughout the water column from in winter, except no detection of GSH in 80 m.

This work also studied the concentrations of Cys, GSH and NAC in extracted Lake Biwa fulvic acids

(LBFAs) for the first time (at 0 m: cysteine, 0.8 nM; glutathione, 1.6 nM; NAC, 2.5 nM; at 10 m: cysteine, 1.4 nM; glutathione, 0.6 nM; NAC, 1.6 nM) (Fig. 2). The concentrations of Cys (0.9 nM), GSH (1.4 nM), and NAC (2.4 nM) were also measured in the dissolved phase of standard FAs provided by JHSS (Fig. 3). The ratios of dissolved to particulate thiols on an average was observed for Cys (1.40% in summer; 2.59% in winter) and GSH (1.66% in summer; 2.39% in winter)

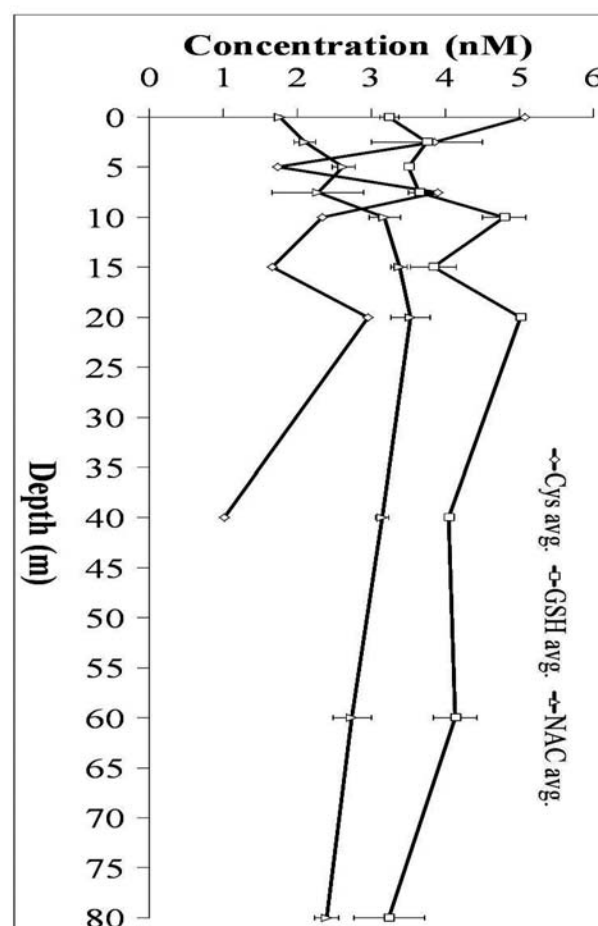


Fig. 1 The average concentrations of dissolved cysteine (Cys) (diamond shape), glutathione (GSH) (square shape) and N-acetyl-L-cysteine (NAC) (triangle shape) in the vertical water profiles (0 m-80 m in depth) of Lake Biwa in summer. Cys was not detected in the depth of 60 m and 80 m. The concentrations of dissolved Cys, GSH and NAC in Lake Biwa were detected in 2-6 nM, 2.7-5.1 nM and 1.6-4 nM, respectively. The replicated samples for total dissolved Cys, GSH and NAC had average relative standard deviation for concentrations of 64%, 9% and 11%, respectively; and, the standard error ranges for the dissolved Cys, GSH and NAC were shown in here.

These results revealed that particulate thiols transformed to dissolved thiols at high ratio in winter rather than in summer. Relatively smaller proportions of particulate Cys (<0.35% at 0 m; <0.65% at 10 m) and GSH (<0.75% at 0m; <0.22% at 10 m) residues were remained in the FAs of Lake Biwa. The source of NAC in dissolved thiols might be wastewater, as dissolved NAC (2 nM) was found in wastewater while no detection of NAC in particulate. The particulate Cys and GSH were significantly covariant, and the ratios between them reflected the differences in the plankton community composition and availability of these compounds. Availability of thiols in Lake Biwa might be

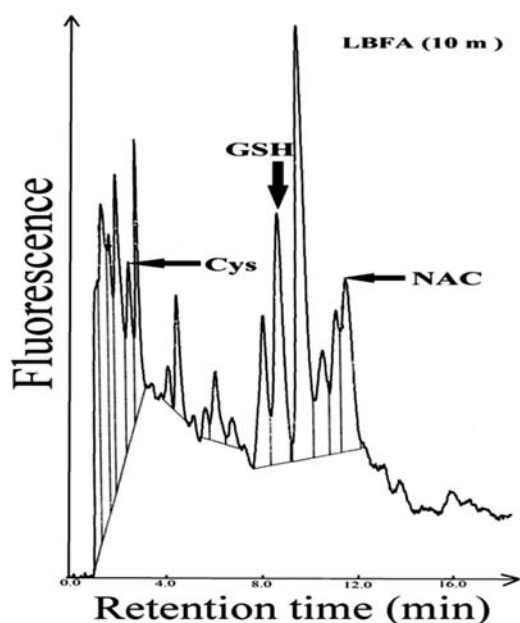


Fig. 2: The chromatogram of the extracted Lake Biwa fulvic acid (LBFA) in the depth of 10 m at T1 station of Lake Biwa. Thiols: Cysteine, Glutathione and N-acetyl-L-cysteine were found in the LBFA.

influenced by other environmental factors especially, chlorophyll abundant region. The nM to sub-nM of both particulate and dissolved thiols in Lake Biwa indicates the existence of an important class of organic ligands with chalcophile metals (Cu, Cd, and Zn), which were low in Lake Biwa.

Reference: Rasheduzzaman Md, Kawaguchi M, Obata H, Maruo M (2018) Determination of Dissolved and Particulate Thiols in Lake Biwa Water and Extracted Fulvic Acids by Solid Phase Extraction Followed by HPLC with Fluorescence Detection. *Limnology*, 19, 299-309.

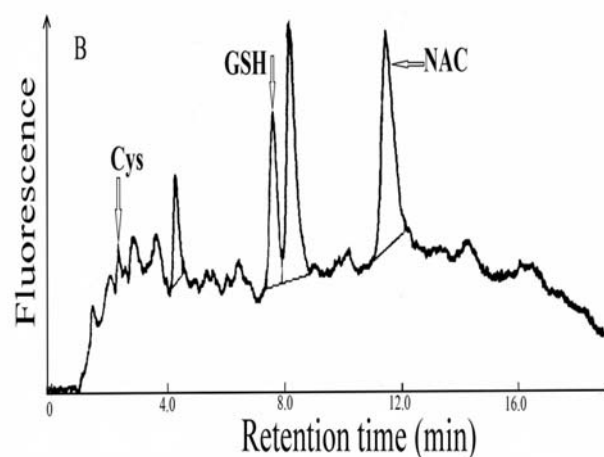


Fig. 3: Chromatogram for the identification of the fulvic acid bearing Cys, GSH, and NAC in the standard Lake Biwa fulvic acid (LBFA) that was purchased from the Japan Humic Substances Society (JHSS). The concentrations of Cys, GSH and NAC of the standard LBFA were found very close to the thiols concentrations of the extracted fulvic acid in our laboratory.