Molecular insights into reversible redox sites in solid-phase humic substances as examined by electrochemical in situ FTIR and two-dimensional correlation spectroscopy

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ABSTRACT

Solid-phase humic substances (also termed humins, HMs) are the largest fraction of humic substances (HSs) in soils and sediments, which are recently shown to be capable of mediating electron transfer in many key biogeochemical processes. However, the redox properties of HMs remains poorly investigated, likely due to their structural complexity and the lack of efficient methodology. Herein, an electrochemical in situ FITR spectroscopic (EC-FTIRS) technique coupled with two-dimensional correlation spectroscopy (2D-COS) was employed for the first time to study the redox process of complex HMs at a molecular level. In situ FTIR identified that the IR bands of quinone and phenolic moieties in the HMs were potential dependent, suggesting that they were the main redox sites in response to the redox transformation of the HMs. Meanwhile, In situ FTIR characterizations showed that the significant variations in IR bands positioned at ~1500 and ~1470 cm^{-1} in response to the applied potentials, providing evidence for the presence of quinone radical and dianion intermediates during the redox process. The 2D-COS analysis was used to further explore variations in infrared intensities as a function of the potentials applied to reduce or oxidize the HMs, which indicated the occurrence of the typical redox reactions of quinone in the HMs. These results improve our understanding on the redox mechanism of HMs at the molecular level and have significant implication for in-depth understanding of biogeochemical processes.

1. Introduction

Redox reactions play a key role in biogeochemical processes in the earth surface system, which link the global carbon cycle, climate change, transformation and fate of organic and inorganic substances, and soil fertility (Borch et al., 2010; Zhu et al., 2017). Humic substances (HSs), important components of natural organic matter, have been shown to be capable of facilitating the electron transfer in these bacteria-driven biogeochemical processes due to their unique redox properties (Lovley et al., 1996; Scott et al., 1998; Aeschbacher et al., 2010; Nurmi and Tratnyek, 2011; Piepenbrock et al., 2014). Numerous previous studies have worked on the redox properties of HSs, revealing that functional moieties such as phenolic, quinone, and N- and S-containing groups contributed to the redox activity of HSs (Scott et al., 1998; Aeschbacher et al., 2010, 2011; Klüpfel et al., 2014b). HSs facilitated bacterial electron transfer has attracted great attention in recent years, but studies have mainly focused on the redox properties of soluble HSs (Scott et al., 1998; Nurmi and Tratnyek, 2011).

HSs are operationally divided into soluble humic acids (HAs) and fulvic acids (FAs) and solid humins (HMs) depending on the extraction procedure from the soil (Sutton and Sposito, 2005). As above-mentioned, the redox properties of soluble HAs and FAs have been extensively explored, whereas the dissolved humic pool is relatively small in natural environments. Instead, HMs, the solid-phase HSs that is not water-soluble under any pH conditions are the largest fraction of HSs, typically representing more than 50% of organic carbon in soils (Lau et al., 2015; Simpson et al., 2007). HMs have also been displayed remarkable redox activity for facilitating microbial electron transfer process in natural environments. They have been shown to be capable of serving as redox mediators to promote bioreduction of Fe(III) in sediment, facilitate microbial dehalogenation of pentachlorophenol and debromoniation of tetrabromobisphenol A, and regulate anaerobic

carbon mineralization and suppress CH_4 production in peatland eco-systems (Roden et al., 2010; Zhang and Katayama, 2012; Zhang et al., 2013). Despite the importance of HMs as electron mediators in these processes, the redox properties of HMs remains poorly characterized, mainly due to its limited solubility, structural complexity and the lack of efficient methods. Measurement techniques, such as electron spin resonance and ex situ FTIR, have been previously used to explore the redox nature of HMs, but these approaches are only able to provide information on the electronic characterization and molecular structure (Zhang and Katayama, 2012). Experiments are required, therefore, to explicitly link functional groups to redox transition of HMs with advanced techniques, which can define the contributions of primary functional groups within HMs to their redox activity, and subsequently provide fundamental information on redox active HMs.

Electrochemical in situ FTIR spectroscopy (EC-FTIRS) is a technique that is capable of revealing the chemical nature of solid or solution species in response to electrochemical redox reactions (Ye et al., 2016). Hence, EC-FTIRS has been frequently used to probe redox interfacial processes of inorganic catalysts, polymers, biomolecules and even bacterial cells, providing a more realistic picture on the electron transfer of the redox species (Ataka et al., 2004; Zhou et al., 2010; Kellenberger et al., 2012; You et al., 2015). This unique feature makes this technique well-suited for in situ studies of HMs. It is noteworthy that traditional one-dimensional FTIR spectra often suffer in overlapping problems and are unable to display the sequential order of functional group changes involved in redox reactions. Two-dimensional correlation spectroscopy (2D-COS), a versatile analysis technique on the base of conventional spectra in response to an external perturbation, can potentially overcome these shortcomings (Chen et al., 2015). The 2D-COS has also been applied to interpret spectroelectrochemical data (e.g. UV/vis, Raman and NMR) under an external perturbation, such as potential and electrolysis time (Ambrosio and Gewirth, 2010; Boisseau et al., 2015; Kim et al., 2004). In this regard, we applied 2D-COS along with EC-FTIRS to characterize the redox functional groups of the HMs. The use of 2D-EC-FTIRS-COS is expected to clarify the redox properties of HMs.

Herein, we used the 2D-EC-FTIRS-COS to systematically probe variations in chemical structure of HMs under different redox conditions. To the best of our knowledge, 2D-EC-FTIRS-COS has not been used to investigate redox properties. To this end, HMs extracted from three type of soils were used as the representative solid-phase HMs. Electrochemical measurements indicated that the extracted HMs harbor redox-active moieties. The EC-FTIRS technique and 2D-COS FTIR spectra revealed the change in the functional groups and sequence changes in response to the redox transformation of HMs. This study gained a direct molecular-level insight into the electron-mediating nature of HMs.

2. Materials and methods

2.1. Chemicals and materials

HM materials were extracted from a forest soil in Jilin Province, China, an upland soil in Yunnan Province, China, and a paddy soil in Guangzhou, China. Hexa-ammineruthenium (III) chloride (Ru^{3+}), 1,1′-ferrocenedimethanol (Fc), and 1,1′-ethylene-2,2′-bipyridyldiylium dibromide (Diquat) were purchased from Sigma-Aldrich. 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS) was purchased from Aladdin. Millipore water (18 MΩ·cm) was used to prepare all aqueous solution. Chitosan powders were dissolved in HCl to make a chitosan solution (1%, pH 5.5).

2.2. Extraction of HMs

HMs extracted from three soils (forest, upland and paddy soils) in Jilin, Yunnan and Guangdong province, China, designated as JLHM,

YNHM and GDHM, respectively, were used for most of the experiments in this study. To extract HMs from these soil samples, 100 g air-dried soils were treated with 150 mL 2% hydrofluoric acid for 48 h to remove silicate. The mixture solution was centrifuged at $12,000 \times g$ to discard the supernatant. This procedure repeated three times in order to completely dissolve the inorganic solids. The residue was further treated for several times with 0.1 M NaOH and 2 M HCl, respectively, until the dark colour in the supernatant was not obvious (Zhang and Katayama, 2012). Then, the residue was thoroughly rinsed with distilled water to remove all chloride ions and freeze-dried. Heat treatment was used to remove the organic matter in HMs, which was also conducted by heating 1.0 g HMs in a muffle furnace at 600 °C for 1 h. The resulting product was termed heat-treated HM, which was considered as the organic-burnt control material in the electrochemical and in situ FTIR tests. In addition, the HMs were also treated with HCl and hydroxylamine hydrochloride ($NH_2OH \cdot HCl$), respectively, which was used to remove the HCl-extractable labile metals and amorphous Fe(III) oxide, respectively (Zhang and Katayama, 2012). There treatments were conducted by dispersing 1.0 g of JLHM in 0.1 M HCl and 0.1 M hydroxylamine hydrochloride ($NH_2OH \cdot HCl$), respectively, which were shaken at 150 rpm for 24 h. The precipitate was collected by centrifugation and washed with distilled water. The resulting products were termed HCl- and $NH_2OH \cdot HCl$-treated HMs, respectively.

2.3. Electrochemical measurements

Cyclic voltammograms (CVs) were recorded by a CHI660D workstation (Chenhua Co. Ltd., China), where a glassy carbon (GC) electrode was used as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a spiral Pt wire as the counter electrode, respectively. The working electrode was prepared by coating the HM-chitosan film on the GC electrode (Kim et al., 2012; Kim et al., 2014). To prepare the HM film electrodes, HM solids were dispersed into water by sonication. Then, the suspension (5 mg/mL) was mixed with a chitosan solution to make HM-chitosan suspension. Aliquots (15 µL) of these HM-chitosan suspensions were spread onto the GC electrode and dried under vacuum. After neutralized in 0.1 M of phosphate buffer solution (PBS, pH 7.0), an insoluble hydrogel film was formed on the GC surface. The CVs of the HM electrodes were performed in 0.1 M PBS (pH 7.0) with 50 µM Ru^{3+} and 50 µM Fc at a scan rate of 5 mV/s. The amplification ratios (AR) of the currents for the Fc oxidation and Ru^{3+} reduction processes on the HM electrode at various scan rates were calculated as previously reported (Kim et al., 2017). The electron transfer capacity of HMs, including electron-accepting capacity (EAC) and electron-donating capacity (EDC), were evaluated by mediated electrochemical reduction and oxidation methods (Aeschbacher et al., 2010). The electrochemical measurements were also performed by using a conventional three-electrode cell system. A glassy carbon cylinder with a volume of 15 mL was used both as the working electrode and electrochemical reaction vessel, a SCE as the reference electrode, and a spiral Pt wire as the counter electrode, respectively. The glassy carbon cylinder was filled with 10 mL of PBS (0.1 M, pH 7). Amperometric measurements were performed by applying a potential of −0.49 V or +0.61 V vs standard hydrogen electrode (SHE) to the working electrode for measuring the EAC and EDC of the HMs, respectively. After the constant background currents were achieved, 10 mM stock solutions (120 µL) of the electron transfer mediators (Diquat for the EAC measurements or ABTS for the EDC measurements) were added to the cells, respectively. After the constant background currents were obtained once again, small amounts (i.e., 50 µL) of the HM suspensions were added to the glassy carbon cylinder cell. The resulting peak currents were integrated to calculate the EAC and EDC values.