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Application of selective H/D exchange coupled to Fourier Transform Ion Cyclotron Resonance mass-spectrometry to investigation individual compartments of Humic Substances

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Abstract Fourier Transform Ion Cyclotron Resonance Mass spectrometry (FTICR MS) is widely used to explore molecular space of humic substances. However, it cannot easily distinguish between structural isomers. We have developed an approach of selective H/D exchange (HDX) coupled to FTICR MS, which provides information about C-H bonds of individual species. This study demonstrates feasibility of comparing structures with common molecular formulas identified in HS from different sources. We believe that combination of different selective isotope labeling techniques will enable identification of individual molecular compartments of natural HS

Introduction

Humic substances (HS) are natural organic compounds that are ubiquitous throughout the environment. They are characterized with non-stoichiometric elemental compositions and extreme structural heterogeneity, which provides for their recalcitrant character and multiple life-sustaining functions (MacCarthy, 2001). This molecular heterogeneity hinders greatly structural analysis of humic materials. One approach to solve this problem is to use Fourier Transform Ion Cyclotron Resonance Mass spectrometry (FTICR MS), which due to its high resolution and mass accuracy, allows one to observe almost all ionizable molecules in the HS ensemble (Hertkorn et al., 2007). However, its major limitation is non-selectivity to structural isomers, which makes it impossible to identify the exact molecular constituents of humics and consequently to fully compare HS of the similar origin.

Selective isotopic exchange can overcome this limitation because structural isomers are likely to differ in the total number of exchangeable atoms. Stenson and co-workers (2014) were able to verify isomeric complexity of HS using reversed-phase chromatography followed by H/D exchange (HDX) of isolated ions directly at mass-spectrometer. However, these experiments are time consuming and, therefore, only a small fraction of HS can be investigated.

Recently, we have developed a simple method of exhaustive exchange of mobile protons in natural organic matter (Kostyukevich et al., 2014). FTICR MS was used to monitor HDX and calculate H/D series for the most of detectable molecular ions. Our intention was to develop this approach further by using selective isotopic exchange of skeletal protons to explore information about C-H bonds of different species. Previously, we have demonstrated that deuterium labeling using basic and acidic catalysis coupled to FTICR MS enabled determination of exact structures of molecular components of humic-like substances synthesized from the well known monomers (Zherebker et al., 2015). In this study, we showed that combination of different selective HDX allows to distinguish different isomers in various HS isolated even from the similar source.

Materials and methods

Coal hymatomelanic acids (CHM) from 2 leonardites were used for this study. That samples were assigned as Leonardite 1 and 2. H/D exchange of skeletal protons were performed by the adapted procedure (Zherebker et al., 2015): 300 μl of 4 M NaOD or 16% DCl in D2O were added to 5 mg of CHM and heated at 120 °C for 40 hours in a sealed tube. Then the samples were desalted using PPL SPE (Dittmar et al., 2008). Elution was carried with methanol and final solutions were used for MS analysis. HDX of mobile protons was conducted by a twofold dilution of the methanol solution with D2O.

The samples were analyzed using Bruker Daltonics 12T Apex Qe FTICR-mass spectrometer housed at the College of Sciences Major Instrumentation Cluster (COSMIC), Old Dominion University, VA. Electrospray ionization (ESI) in a negative mode was used in these studies. Prior to analysis, the instrument was externally calibrated using polyethylene glycol standard. The samples were injected at a flow rate of 120 μL h−1 using an Apollo II ESI ion source

The FTICR MS data were processed using the lab-made “Transhumus” software based on the total mass difference statistics algorithm (Kunenkov et al., 2009).

Results and Discussion

The typical mass spectrum of the coal hymatomelanic acid (CHM) comprises about 7000 peaks. Negative ion ESI FT-ICR MS resolves 5300 CcHhOoNnSs molecular formulas, the majority of which is CHO. The assigned formulas were plotted into Van Krevelen diagram, which enables segregation of molecular space into different chemical classes based on H/C and O/C atomic ratio. Figure 1 shows that condensed hydrocarbons and lignin-like compounds constitute the major part of CHM, which is in agreement with the literature.

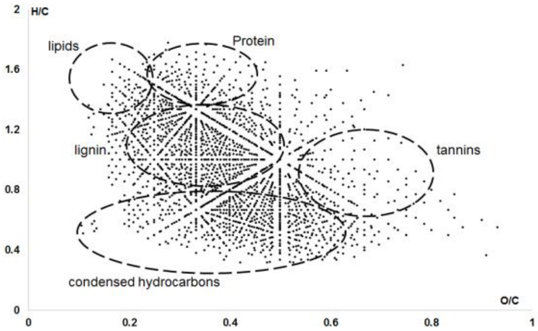
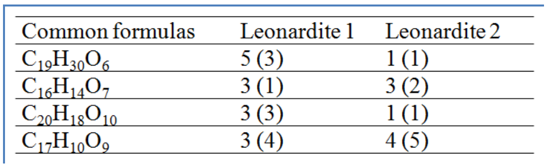


Fig. 1 Van Krevelen diagram plotted from the FTICR MS data for CHM with highlighted regions of humic precursors.

Typically, HS from the similar sources possess a large amount of common formulas. However, this overlap may hide different structures. To determine the true and false similarities of identifications we performed selective H/D exchange, which provides information about C-H bonds needed to compare different samples. Solution acid/base catalyzed HDX was applied for comparison CHM isolated form 2 leonardites, which possess more than 70% common molecular formulas. Table 1 shows lengths of HDX series under base and acidic catalyses in those two cases.

Table 1 The length of NaOD and DCl (in brackets) catalyzed HDX series for some common formulas of CHM isolated from Leonardite 1 and 2



Common formulas	Leonardite 1	Leonardite 2
$C_{19}H_{30}O_6$	5 (3)	1 (1)
$C_{16}H_{14}O_7$	3 (1)	3 (2)
$C_{20}H_{18}O_{10}$	3 (3)	1 (1)
$C_{17}H_{10}O_9$	3 (4)	4 (5)

It could be seen that HDX allows for distinguishing among common formulas without preliminary separation and tandem mass-spectrometry. However, a use of one labelling technique may lead to false interpretation as in the case of $C_{16}H_{14}O_7$. In general, HDX strongly depends on the position of substituents. DCl catalyzed HDX leads to labelling of aromatic protons exclusively. Therefore the length of HDX indicates the degree of ring substitution. According to that aromatic moieties of Leonardite 2 are less substituted than Leonardite 1 within lignin region on Van Krevelen diagram. This could be particularly important for consideration of coal formation from peat, which properties strongly depend on lignin structure.

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