STRUCTURAL STUDY OF DISSOLVED ORGANIC MATTER ISOLATED FROM SURFACE WATER AND PERMAFROST SOURCES OF KOLYMA RIVER BASIN (NORTH-EASTERN SIBERIA, RUSSIA)

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ABSTRACT: Four dissolved organic matter (DOM) samples isolated from riverine and lake water, permafrost meltwater stream and ice in Eastern-Siberian Arctic were characterized using ^{1}H NMR spectroscopy. A structural marker calculated from ^{1}H NMR data was proposed for quantifying transformation degree of DOM as a ratio of alkyl long chain protons to alkyl protons of the short bridge-like structures located in α-position to carbonyl and carboxyl groups or aromatic rings.

KEYWORDS: Arctic, DOM, ^{1}H NMR, molecular markers, permafrost

INTRODUCTION: Being formed under permafrost conditions Arctic ecosystems are supposed to be highly sensitive to the global warming processes. At that, permafrost represents a global reservoir of organic carbon due to its very slow degradation rate under low-temperature conditions. So, considerable changing in biogeochemistry processes in Arctic ecosystems because of permafrost thawing and organic carbon release should be expected. In the first place it could be caused by increase of released organic carbon bioavailability (Uhlířová et al., 2007; Holmes et al., 2008). Structural studies of Arctic natural organic matter using modern analytical techniques (e.g. Woods et al., 2011) are of great importance in light of distinctive molecular markers search. This aim achievement would allow to carry out organic carbon release monitoring and obtain more exact estimations of the global carbon cycle under the contemporary climate warming conditions. Thus, 15 bacteriohopanepolyoles contained in Arctic river and estuarine sediments were analyzed using LC-MS technique and demonstrated to be aplicable as biomarkers to examine the transportation of terrestrial natural organic matter to the Arctic Ocean, though these compounds were not specific only for permafrost ecosystems (Cooke et al., 2009). So, the objective of the presented work was a structural study of Arctic DOM originated from surface water and permafrost sources using ^{1}H NMR spectroscopy.

MATERIALS AND METHODS: The DOM sampling took place in July 2012 during the Polaris expedition into the Kolyma river basin (East-Siberian Arctic, Russia). The materials studied in this work are described in Table 1. Two DOM samples were obtained from surface freshwater sources and two others – from permafrost ice and meltwater. All the materials were isolated using Amberlite XAD16 resin according to the standard procedure (Aiken et al., 1992) and transported to the Lomonosov Moscow State University. The samples sorbed on XAD16 resin were eluted with 0.1 M NaOH. The obtained alkaline extracts were passed through the cation-exchanging resin Amberlite 120R in H-form (Rohm and Haas Ltd.). The desalted samples were dried in the vacuum oven at 40°C, and then stored at −15ºC in the darkness.

Table 1. DOM samples description.

DOM sample cipher	DOM sample source
OML	Omolon river near its confluence with the Kolyma river, from the depth of 3 m
SCH	Schuchie lake, from the surface
DYS	Permafrost meltwater stream at Duvanny Yar ravine, Kolyma riverbank
DYI	An ice wedge included in permafrost at Duvanny Yar ravine, Kolyma riverbank

Proton NMR spectra were acquired using Avance-400 NMR spectrometer (Bruker) operating at 400 MHz proton frequency. A weight of ~10 mg of a DOM sample was placed into 5 mm NMR tube, attached to vacuum line, and dried about 6 hours at 10^{-2} Torr for complete removing of water. The dry sample was then dissolved in 0.7 ml of anhydrous DMSO-d6 (Merck, 99.95 isotopic purity) under vacuum conditions. The spectra were acquired using 90° pulses, relaxation delay of 2 s, about 100 scans for each spectrum were recorded. Fourier transformation, phase correction and integration of the spectra were performed using MestReC software. A signal of residual protons of DMSO located at 2.5 ppm was used as a reference for proton assignments. The integration of spectral intervals was conducted according to the following assignments (ppm): 0.5-2.05 – protons of alkyl chains (CH_n); 2.05-2.45 and 2.55-3.2 – protons of alkyl chains located in α-position to carbonyl, carboxyl groups or aromatic rings (α-CH_n); 3.2-6.0 – protons attached to O or N substituted carbon atoms or to the corresponding heteroatoms (CH_n-O(N)**H** and C**H**$_n$-O(N)); 6.0-11.0 – aromatic and phenolic protons (arom-OH and arom-H); 11.5-15.0 – protons of carboxyl groups (COOH).

RESULTS AND DISCUSSION: The spectra acquired were characterized by broad partly overlapping bands typical for NMR spectra of DOM and humic substances (Figure 1). All the spectra demonstrated the presence of well resolved and intense resonances in the range of aliphatic protons (0-6 ppm). Contrary to this, spectral bands in the range of aromatic hydrogen (6-11 ppm) were often vastly overlapped with the nearby regions of carboxylic protons or hydrogen atoms of alkoxy groups. The spectra of DOM samples originated from permafrost meltwater and, especially, permafrost ice were characterized by sharp signals of alkyl protons that were absent or weak in the spectra of riverine and lake DOM and indicated low-molecular structures undecomposed under permafrost conditions. Thus, signals at 0.86 and 1.5 ppm could be ascribed to isoleucine δ- and γ-protons; peaks at 3.9 and 8.3 ppm correspond properly to glycine hydrogen atoms attached to C and N correspondingly. The next sharp peaks were presented in all the spectra acquired: a signal about 1.24 ppm indicates methene units of alkyl chains; a triplet at 7.0-7.3 ppm apparently belongs to protons of ammonium groups split on quadrupole ^{14}N; weak peaks at 7.9 and 8.3 ppm could be ascribed to NH groups of aminoacids; and, at last, a remarkable peak at 2.5 ppm corresponds to residual hydrogen of DMSO. Percentages of typical structural units of Arctic DOM calculated from its ^{1}H NMR spectra are presented in Table 2. The overrated value of aromatic hydrogen contribution obtained for the DYS sample is caused by its residual moisture that is confirmed by total overlapping of spectral regions of aromatic and carboxylic hydrogen atoms. The most profound structural feature intrinsic to all samples studied is high content of alkyl-chain hydrogen atoms belonging most probably to hopanes and protein side chains preserved within the permafrost DOM. It leads to significant excess of these protons over α-CH_n protons located in the nearby region. This feature of Arctic DOM allowed us to introduce a use of CH_n/α-CH_n ratio calculated from ^{1}H NMR spectrum for characterizing transformation degree of molecular complex of DOM. A meaning of this parameter was demonstrated by its calculation for the samples studied (high latitude low transformed DOM) and for two standard IHSS samples isolated from the Suwannee River (low latitude highly transformed DOM). The corresponding data are shown in the right side column of Table 2. It can be seen that Arctic DOM differs according to its origin. It is characterized by a value of CH_n/α-CH_n ratio from 1.4 (riverine DOM) to 2.2 (the least degraded DOM originated from permafrost ice) while for the Suwannee River DOM and Fulvic Acids this value comes to 0.7-0.8.

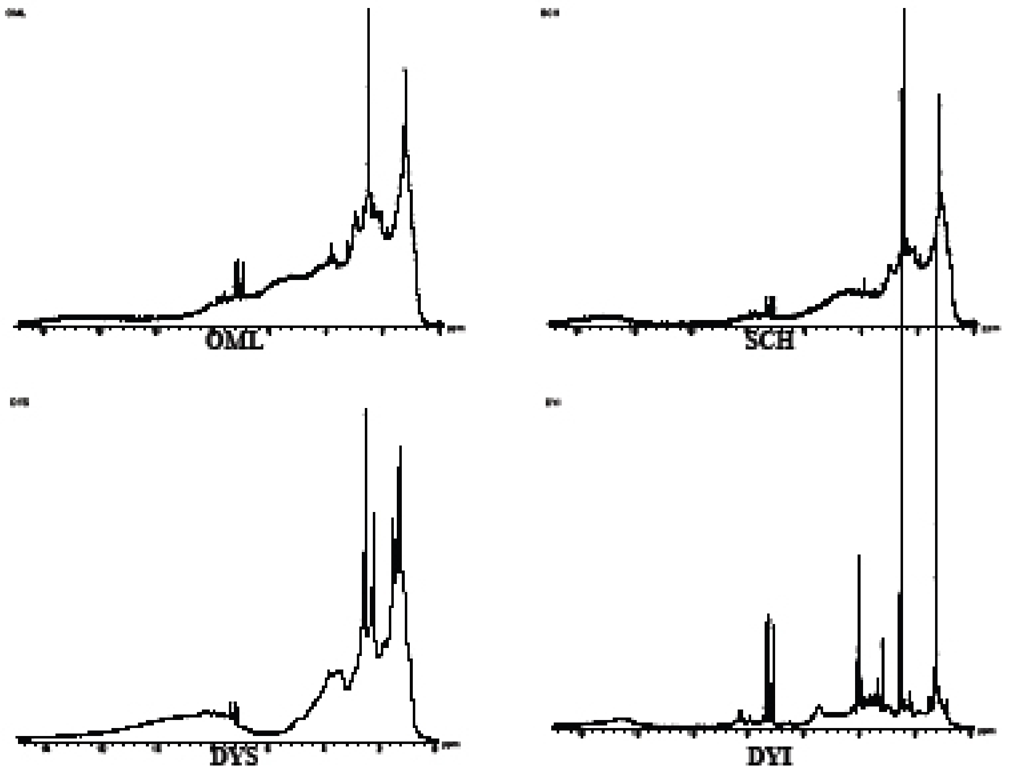

Figure 1. ^{1}H NMR spectra of Arctic DOM isolated from: riverine water (OML); lake water (SCH); permafrost meltwater (DYS); and permafrost ice (DYI). All the samples were dissolved in deuterated DMSO, the signal of residual protons of the solvent are located at 2.5 ppm.

Table 2. Percentages of typical structural units of DOM and $CH_n/\alpha\text{-}CH_n$ values calculated from ^1H NMR data. *SR-DOM* and *SRFA* are DOM and Fulvic Acids, respectively, standard IHSS samples isolated from the Suwannee River.

DOM	Percentages of typical structural units of DOM					CH_n/
	CH_n	α-CH_n	$CH_nO + CH_nOH$	H(OH)-arom	COOH	α-CH_n
OML	28.6	21.0	29.7	11.0	8.8	1.4
SCH	35.9	21.7	27.2	8.7	6.5	1.7
DYS	35.1	18.3	21.3	20.2	4.3	1.9
DYI	26.4	12.1	38.5	14.3	8.8	2.2
SRDOM	*16.5*	*23.1*	*30.8*	*23.1*	*6.6*	*0.7*
SRFA	*21.7*	*26.1*	*23.9*	*15.2*	*13.0*	0.8

CONCLUSIONS: Aplication of ^1H NMR for structural characterization of DOM isolated from different sources in Eastern-Siberian Arctic (Russia) allowed us to introduce a value of $CH_n/\alpha\text{-}CH_n$ ratio calculated from ^1H NMR data for characterizing transformation degree of molecular complex of DOM. Application of this parameter to high and low latitude DOM showed striking differences in structural features of the corresponding molecular ensembles which were enriched with long-range alkyl chains in case of Arctic DOM ($CH_n/\alpha\text{-}CH_n$ ratio = 1.4-2.2 for the samples studied) and depleted with those structures, but rich in short, highly substituted aliphatic fragments in case of the Suwannee River DOM ($CH_n/\alpha\text{-}CH_n$ ratio = 0.7-0.8).

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