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Detection and Structural Identification of Dissolved Organic Matter in Antarctic Glacial Ice at Natural Abundance by SPR-W5-WATERGATE ^{1}H NMR Spectroscopy

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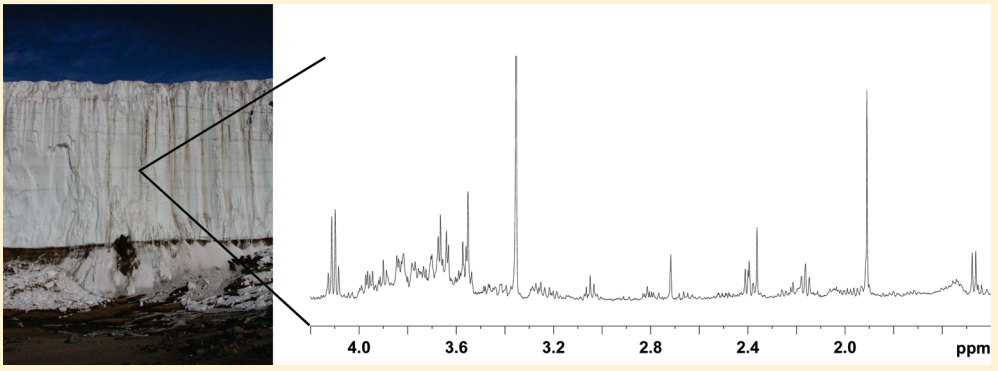
Supporting Information

ABSTRACT:



Dissolved organic matter (DOM) is ubiquitous in aquatic ecosystems and is derived from various inputs that control its turnover. Glaciers and ice sheets are the second largest water reservoir in the global hydrologic cycle, but little is known about glacial DOM composition or contributions to biogeochemical cycling. Here we employ SPR-W5-WATERGATE ^{1}H NMR spectroscopy to elucidate and quantify the chemical structures of DOM constituents in Antarctic glacial ice as they exist in their natural state (average DOC of 8 mg/L) without isolation or preconcentration. This Antarctic glacial DOM is predominantly composed of a mixture of small recognizable molecules differing from DOM in marine, lacustrine, and other terrestrial environments. The major constituents detected in three distinct types of glacial ice include lactic and formic acid, free amino acids, and a mixture of simple sugars and amino sugars with concentrations that vary between ice types. The detection of free amino acid and amino sugar monomer components of peptidoglycan within the ice suggests that Antarctic glacial DOM likely originates from *in situ* microbial activity. As these constituents are normally considered to be biologically labile (fast cycling) in nonglacial environments, accelerated glacier melt and runoff may result in a flux of nutrients into adjacent ecosystems.

INTRODUCTION

Determination of the concentration, composition and cycling of dissolved organic matter (DOM) in natural waters is important for the assessment of global biogeochemical cycling of carbon, which is linked to atmospheric carbon dioxide levels.[1−4] Glaciers and ice sheets represent the second largest water reservoir in the global hydrologic cycle[5] and contain dissolved organic carbon (DOC) that may be cycled through glacial ecosystems.[6] Analysis of organic compounds stored in glacial ice cores may provide insight into past climates[7,8] and contribute to the understanding of biogeochemical reactions and organic carbon turnover in glaciated regions.[9−11] In addition, surface runoff from heavily glaciated regions can have a significant impact on DOC concentrations, composition, bioavailability, and biogeochemistry in downstream environments.[12,13] It has recently been determined that glaciers are the source of some of the oldest and most reactive DOM found in adjacent rivers,[5] so alteration of glacial cover driven by climatic change may alter the quantity, age and biogeochemical reactivity of DOM entering nearby watersheds and coastal regions.[5,12,13]

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DOM in sea ice, glacial ice, and nearby watersheds has traditionally been characterized *in situ* by fluorescence spectroscopy because of its high sensitivity,[6,14−18] and when combined with statistical analyses, provides a general classification of glacial DOM (i.e., humic-like, protein-like). However the structure and composition of DOM cannot be elucidated fully by this technique alone. Consequently, the detection of specific structures will provide insights into both the nature of glacial DOM and its potential role in the global carbon cycle.[7,8] Recently, electrospray ionization (ESI) Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry (MS) provided an unprecedented molecular-level characterization of DOM in glacial ice from Russia[8] and Greenland.[11] These studies revealed differences in DOM composition between modern and ancient ice[8] along with seasonal DOC fluctuations in both supraglacial (at the glacier surface) and subglacial (at the glacier base) meltwaters.[11] ESI-FT-ICR-MS is a highly sensitive analytical technique and provides high resolution mass spectral information about DOM, however, observed ion intensities may be biased by the matrix in the ionization source.[8,19,20]

Nuclear magnetic resonance (NMR) is often used to study organic matter in the biogeosphere.[21] Solid-state ^{13}C NMR is commonly used to study organic matter in soils and sediments but this technique may require large quantities, as much as ∼100 mg of isolated organic matter, and this can be challenging in samples such as DOM where organic concentrations are often low.[21] Consequently, solution-state NMR has emerged as a powerful nonselective approach for the determination of the structural composition of marine[22−24] and freshwater[25−27] DOM, but it also may require the isolation of milligrams of material which may or may not be fully representative of DOM in its natural state. The development of the ^{1}H NMR pulse sequence Shaped PResaturation−WATER suppression by GrAdient-Tailored Excitation using optimized W5 pulse trains (SPR-W5-WATERGATE) facilitates the direct detection and structural determination of unaltered DOM in river, lake, and ocean waters with DOC content as low as ∼1.1 mg/L without any sample isolation or preconcentration.[28] The extension of this technique to glacial ice will allow for the direct detection of this DOM *in situ* without sample alteration.

Here we present a molecular-level characterization of glacial ice DOM by ^{1}H NMR spectroscopy. NMR spectra of unaltered natural ice samples from the Victoria Upper Glacier in Antarctica were acquired by the SPR-W5-WATERGATE ^{1}H NMR spectroscopy pulse sequence to elucidate and quantify the molecular components dissolved in glacial ice. Current and accelerated Antarctic ice loss[29] may result in the release of this material into adjacent watersheds and coastal areas providing newly available DOM substrates. Knowledge of the molecular signature of DOM in glacial ice will aid in the prediction of the potential biogeochemical impacts of melting glaciers on nearby watersheds and coastal systems.

EXPERIMENTAL SECTION

Victoria Upper Glacier. Glacial ice samples were obtained from the Victoria Upper Glacier in the McMurdo Dry Valleys of Antarctica (77°16′S, 161°29′E). This is a cold-based glacier in a polar desert environment adjacent to an ice-covered proglacial lake. The glacier terminates in a ∼50 m high ice cliff, the lower ∼15 m of which consists of "basal" ice which has interacted with the glacier bed.[6,30] Basal ice forms either by the upward flow of pore water through water-saturated subglacial sediments and subsequent freezing onto the glacier sole, or by metamorphism of surface ice, creating ice that is physically and/or chemically distinct from glacier ice that forms by compression and recrystallization of snow initially deposited on the glacier surface (meteorically derived glacier ice).[31] A pro-glacial apron of glacier ice calved from the terminal ice cliff of Victoria Upper Glacier extends to an elevation of ∼10−15 m at the base of the cliff providing access for sampling.[6,30] Three ice types were sampled in January 2003: the uppermost ice layer composed of meteorically derived glacier ice, basal ice formed at the bed of the glacier and ice from the glacier-basal ice contact. Previous analyses of these samples revealed mean DOC concentrations of 8 ± 0.16 mg/L and 3 ± 0.06 mg/L in samples of glacier and basal ice, respectively, and a large DOC concentration (mean ∼12 mg/L) in samples collected within 10 cm of the glacial-basal ice contact.[6] Although the source of the DOC is currently under investigation, the above average concentrations in these ice samples imply sedimentary sources and/or or *in situ* formation.[18] Samples were collected by cutting a trench ∼30 cm deep (∼70 cm high and ∼30 cm wide) into the cliff face across the glacier ice-basal ice contact with a chain saw to remove weathered ice that has been exposed at the surface. Blocks were wrapped, marked for orientation, and stored in coolers for transport to the laboratory at the University of Alberta, where they were stored at −20 °C. Samples were cut from the blocks in a cold room using a band saw and placed in sterile Whirlpak bags. At least 3 cm of ice was removed from the block surface before samples were cut. There was no evidence of sample melting at any point between sample collection and analysis. Ice samples were handled using sterile gloves and in a sterile cold room to avoid any contamination of the samples.

Sample Preparation and NMR Spectroscopy. Ice samples (∼4 g) were allowed to melt into a scintillation vial. Each of the three ice samples was prepared in triplicate for NMR analysis. Immediately after melting, a small amount of NaN_3 (∼5 mg) was added to restrict microbial growth followed by homogenizing the sample by swirling the vial and filtration through a 0.2 μm syringe Teflon filter to remove any fine particulates. 800 μL of filtered melted ice was transferred to 5 mm NMR tubes and 2.5% D_2O (v/v) was added to each sample for the spectrometer lock. Organic free deionized Milli-Q water was used as a method blank to ensure all ^{1}H signals in the spectra were from the ice samples and not background contamination (Supporting Information (SI) Figure S1). All NMR experiments were performed on a Bruker Avance 500 MHz spectrometer equipped with a 5 mm QXI probe with an actively shielded Z-gradient. ^{1}H NMR was performed using the SPR-W5-WATERGATE sequence for water suppression developed by Lam and Simpson.[28] In this sequence, the W5-WATERGATE sequence is preceded by a train of 2000, 2 ms calibrated 180° pulses separated by a 4 μs delay. In practice, this sequence slightly attenuates signals up to 1.1 ppm on either side of the water resonance, with signals <0.4 ppm being completely attenuated.[28] Experiments were acquired with 30 720 scans, a saturation loop of 2.25 s, and 32 768 time domain points with an acquisition time of 1.1 s for a total interpulse delay of 3.35 s. Spectra were apodized by multiplication with an exponential decay producing a 1 Hz line broadening in the transformed spectrum with a zero-filling factor of 2. Spectra were calibrated externally to the trimethylsilyl resonance (0 ppm) of 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt.

Molecular Identification and Quantification. The Bruker Biofluid Reference Compound Database (version 2.0.3, Bruker

BioSpin) in conjunction with analysis of standard compounds facilitated the identification of the constituents within the glacial ice. The pH of all samples was ~6 so the DOM mixture constituents may exist in both their protonated or deprotonated form depending on their pK_a. Therefore, for simplicity all of the compounds identified in this study are reported based on their acidic form. The database contains multiple spectra recorded over a range of pH values. All matches reported are based on the recorded value at pH 6 to best match spectra from the ice samples. Quantification of molecular compounds that did not have overlapping resonances with other constituents was performed by the addition of dimethyl sulfoxide (DMSO; 4.23×10^{-7} mol/L) as an internal standard and Lorentzian deconvolution of the ^{1}H NMR spectra (TopSpin2.0 deconvolution tool, Bruker BioSpin). Each sample (800 μL) was spiked with DMSO and acquired using the same parameters as the samples without the standard. Quantification with a universal reference standard by NMR is highly reproducible when the longest spin−lattice (T_1) relaxation is accounted for[32] and DMSO has been shown to be a convenient internal standard due to its miscibility, nonvolatility and single resonance from six equivalent ^{1}H nuclei in water.[33] The ^{1}H singlet from DMSO at this low concentration resonates at 2.72 ppm likely due to interactions with water molecules. The total interpulse delay was set at 2.5 times longer than the longest measured T_1 of the amino acids and sugars in the samples and corresponds to a magnetization recovery ≥90% between pulses and in turn quantification error arising from incomplete relaxation ≤10%.[32] Formic acid, acetic acid, and MeOH may be slightly underestimated in this study as they exhibited longer T_1 relaxations.

RESULTS

SPR-W5-WATERGATE ^{1}H NMR spectroscopy performed on DOM samples at natural abundance in river, lake, and ocean water resulted in very broad ^{1}H NMR profiles characteristic of complex DOM mixtures arising from multiple sources with various degrees of degradation.[28] In contrast, this technique reveals that glacial ice DOM is composed predominantly of a mixture of small, identifiable molecules, differentiating it from DOM observed in other aquatic environments.[4,23,25,27,28] The water suppression employed by this technique allows for observation of DOM spectral detail (Figure 1A). This method prevents the large ^{1}H water signal from swamping the NMR receiver, suppressing it below the spectrometer noise, which then allows for the direct detection of DOM in unaltered ice samples. Several molecular structures in the DOM from the glacier-basal ice contact sample were identified by comparing the chemical shift and multiplet patterns of the ^{1}H resonances in the spectrum to the NMR spectral database and pure compound standards (SI Figures S2 and S3). Detailed analysis of the ^{1}H NMR spectrum reveals that the glacier-basal ice contact DOM is comprised mainly of free amino acids, small organic acids and biomolecules, simple sugars and amino sugars (Figure 1B, C).

DOM structural assignments in the glacier-basal ice contact sample are proposed based on ^{1}H chemical shift and multiplet splitting patterns. In cases where molecular species only possess a ^{1}H singlet resonance, assignments were carefully based on precise chemical shift matches. The low natural abundance of DOM in glacial ice (~30 h to acquire a ^{1}H NMR spectrum) prevented the acquisition of any multidimensional NMR experiments, however the ^{1}H dispersion of chemical shifts observed in this study permitted the assignment of a range of chemical structures. Based on the presence of several resolved or semi-resolved ^{1}H peaks, several amino acids likely contribute to this mixture. These include alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), aspartic acid (Asp), lysine (Lys), serine (Ser), phenylalanine (Phe) and tyrosine (Tyr; Figures 1 and SI Figure S2). ^{1}H resonances are also present for two amino acid derivatives; pyroglutamic acid (PyroGlu, cyclic lactam of glutamic acid) and norvaline (Nor; Figure 1B). A sharp singlet present at 3.55 ppm likely corresponds to a ^{1}H resonance from glycine (Gly). In addition to amino acids, several other small organic molecules can be identified in the DOM mixture. The dominant species are lactic and formic acid (Figure 1A), with smaller contributions from 3-aminoisobutyric acid, muramic acid and isobutylglycine. Multiple ^{1}H resonances that match a mixture of short chain acids (SCA) are present, likely resulting from a mixture of C_4 to C_{10} acids; however precise structures cannot be deduced due to the similarities in ^{1}H chemical shift patterns of these molecules and are therefore referred to as SCA. In addition, singlet ^{1}H resonances most likely from ^{1}H nuclei in acetic acid, pyruvic acid, and methanol (MeOH) were also identified through spectral pattern matching. Finally, comparison of glacial ice ^{1}H NMR spectra within the 3.1−4.8 ppm region with the ^{1}H NMR spectra of glucose, mannose, and galactose along with their corresponding amino sugars (glucosamine, mannosamine, and galactosamine) suggests that some/all of these constituents may be present (SI Figure S3). Precise assignments for this particular region could not be determined due to spectral overlap; however ^{1}H resonances in this region likely originate from a mixture of sugars or amino sugars with underlying resonances from amino acids.

Comparisons between samples from glacier ice (formed in a supraglacial environment), basal ice (formed in a subglacial environment) and ice from the glacier ice-basal ice contact revealed slight variations in molecular composition (Figure 2). Molecular constituents of DOM from the glacier ice-basal ice contact sample (Figure 1) were also found in both the glacier ice and basal ice with only minor differences. Additional resonances from other molecular species were not observed (Figure 2). The aromatic amino acid ^{1}H resonances of Tyr and Phe that were observed in the glacier ice-basal ice contact sample were absent in both the glacier and basal ice, suggesting that they are either absent from the DOM in these samples, or present at concentrations that are too low to detect. In addition, a comparison of the ^{1}H NMR spectra of all three ice samples suggests that the glacier ice may be depleted in amino acids. ^{1}H resonances from Ala, Gly, and PyroGlu are observed, but those from Lys, Leu, Ile, Val, Ser, and Nor are either absent or could not be resolved adequately from the baseline or adjacent ^{1}H signals (Figure 2A). With the exception of the aromatic amino acids Tyr and Phe, the same molecular constituents were observed in both the basal ice and the ice from the glacier ice-basal contact ice region (Figure 2B, C).

To attempt a more accurate comparison between samples, quantification of the identified DOM molecular constituents with fully resolved ^{1}H resonances using a known amount of DMSO as an internal standard was employed, where peak areas were deconvoluted using a Lorentzian fitting function.[34] Figure 3 illustrates a section of the spectrum from the glacier ice-basal contact sample containing DMSO with its corresponding Lorentzian deconvoluted spectrum. The singlet resonance of DMSO at 2.72 ppm arises from six chemically equivalent ^{1}H nuclei

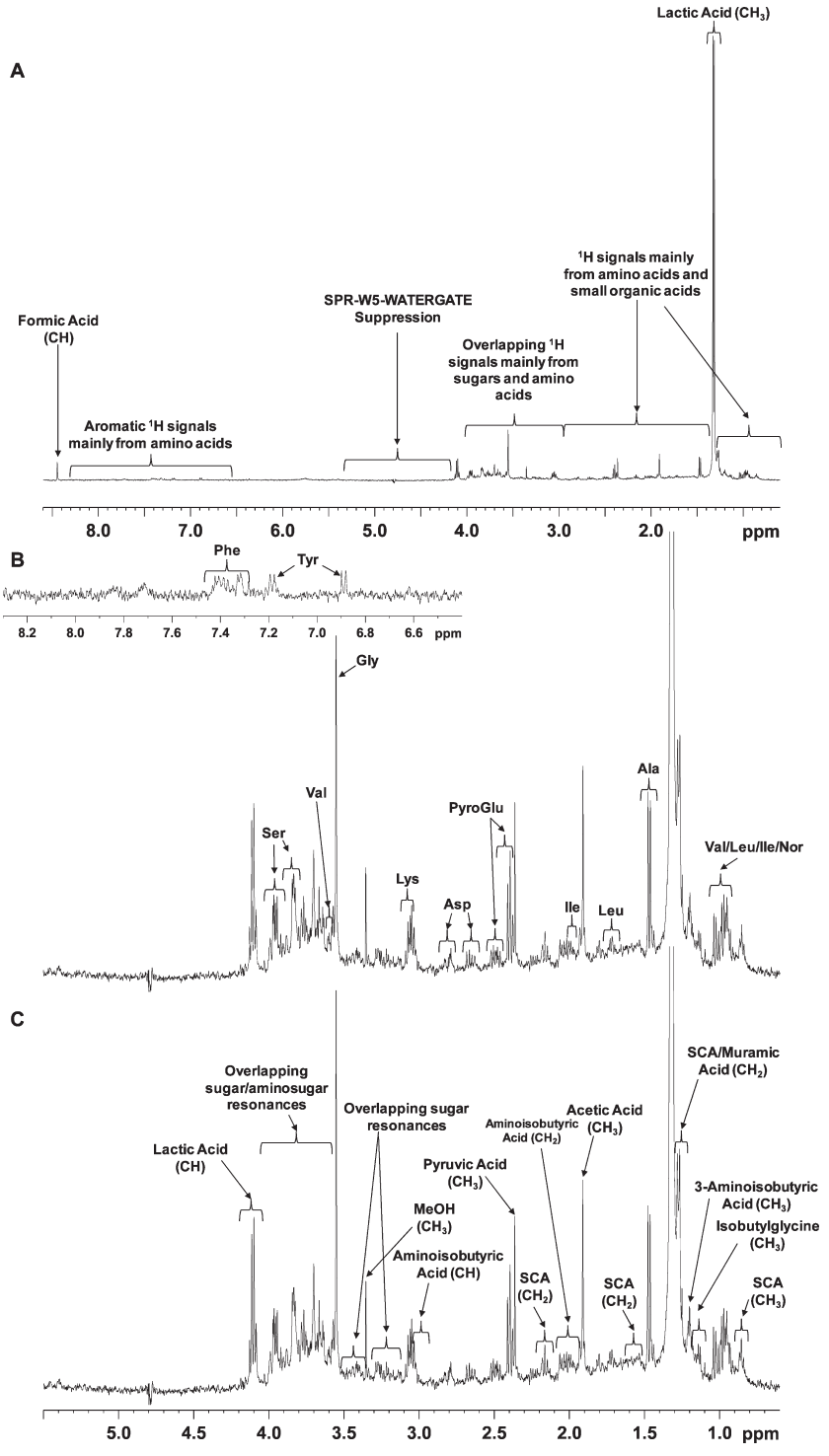

Figure 1. SPR-W5-WATERGATE ^{1}H NMR spectra of Victoria Upper Glacier ice sample from the glacial-basal ice contact, (A) Entire spectrum highlighting the major spectral ^{1}H regions, the suppressed ^{1}H water signal region, along with the lactic and formic acid ^{1}H chemical shift assignments; (B) The same sample spectrum magnified to highlight the resonances assigned to amino acids, inset: aromatic ^{1}H region with amino acid assignments; (C) Magnified spectrum highlighting sugar and amino sugar ^{1}H overlap, and other biologically relevant acid ^{1}H resonances within the ice.

and the area under the peak is proportional to the number of ^{1}H nuclei giving rise to this signal.[34,35] Therefore normalization of peak areas to ^{1}H nuclei allows for the calculation of concentrations of DOM molecular species (see SI for a sample calculation). Generally, the concentrations of the amino acid constituents are highest in the glacier ice and lowest in the basal ice with minor sample differences between concentrations of the remaining constituents (Figure 4). Lactic acid was calculated to be the dominant constituent in all glacial DOM. The quantifiable DOM constituents account for approximately 85% of the DOC content in each of the samples.

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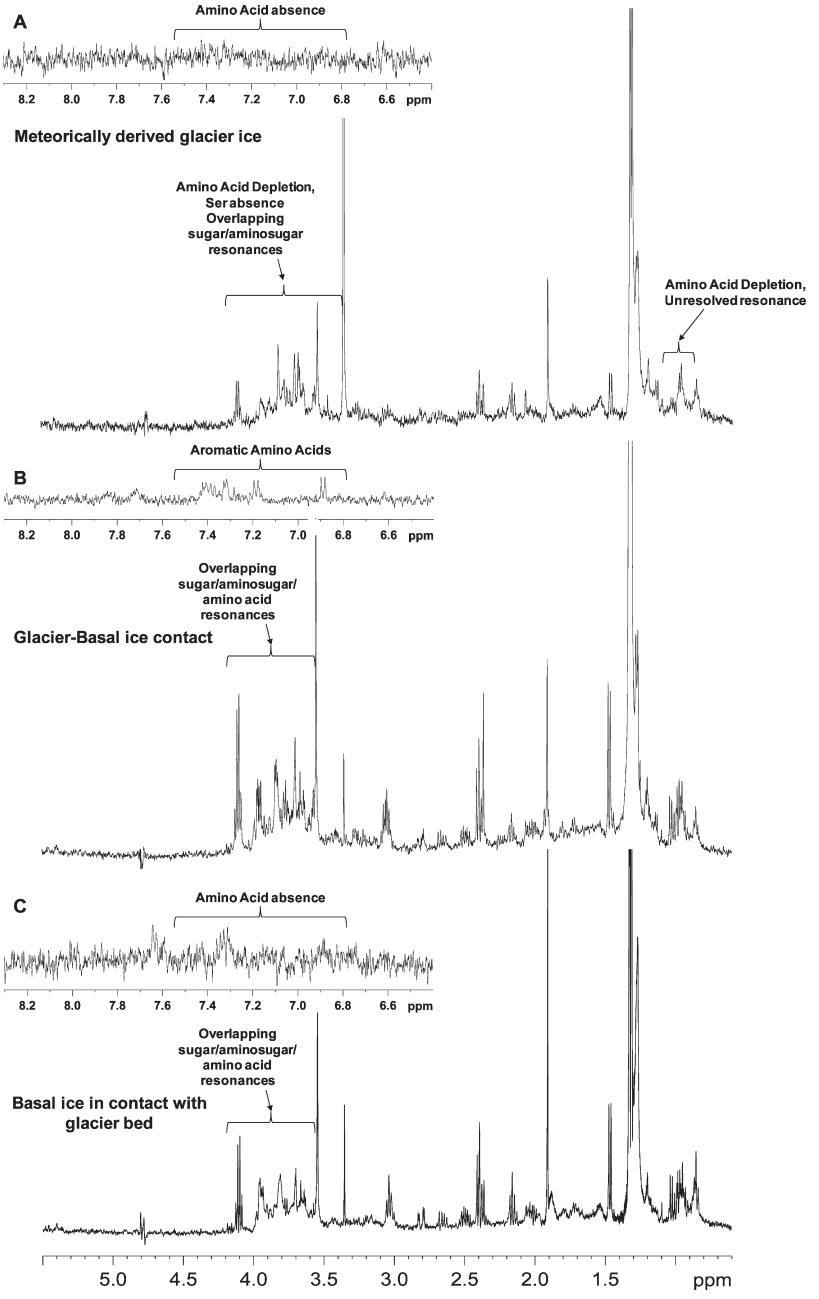

Figure 2. SPR-W5-WATERGATE ^{1}H NMR spectra of Victoria Upper Glacier ice samples, (A) meteorically derived glacial ice, (B) glacial-basal ice contact, (C) basal ice in contact with the glacier bed. Slight variations in amino acid distribution between the ice samples are highlighted.

DISCUSSION

Glaciers are now considered to host diverse ecosystems, and to exert a significant influence on the nature and abundance of nutrients supplied to nearby watersheds.[12,13,36] Supraglacial ecosystems are comprised primarily of bacteria, algae, phytoflagellates, fungi and viruses[36] whereas subglacial ecosystems are dominated by aerobic and anaerobic microbes including chemoheterotrophs, methanogens and anaerobic nitrate/sulfate reducers.[37] It has recently been demonstrated that there is not a minimum temperature for microbial metabolism,[38] which suggests that microbial growth and maintenance can occur within glacial ice. Microbial activity in aquatic ecosystems has been shown to alter and degrade DOM while simultaneously releasing newly synthesized DOM species.[39] It is therefore likely that the molecular species detected in this glacial ice are predominantly of microbial origin.[40] In addition, the similarities between DOM from glacier ice, basal ice, and ice from the glacier ice-basal ice contact suggests a common source of DOM.

Both Gram negative and Gram positive bacteria synthesize the cell wall polymer peptidoglycan that is composed of repeating disaccharide glycan strands that are cross-linked by small peptides.[41] During exponential growth, this polymer is cleaved and subsequently releases its amino acid and amino sugar (including muramic acid) monomer constituents as DOM along with the simple sugars that are associated with the bacterial membranes.[42,43] PyroGlu is an amino acid derivative that has been found in the Archaea protein bacteriorohodpsin.[44] This ^{1}H NMR spectroscopic analysis therefore suggests that Antarctic

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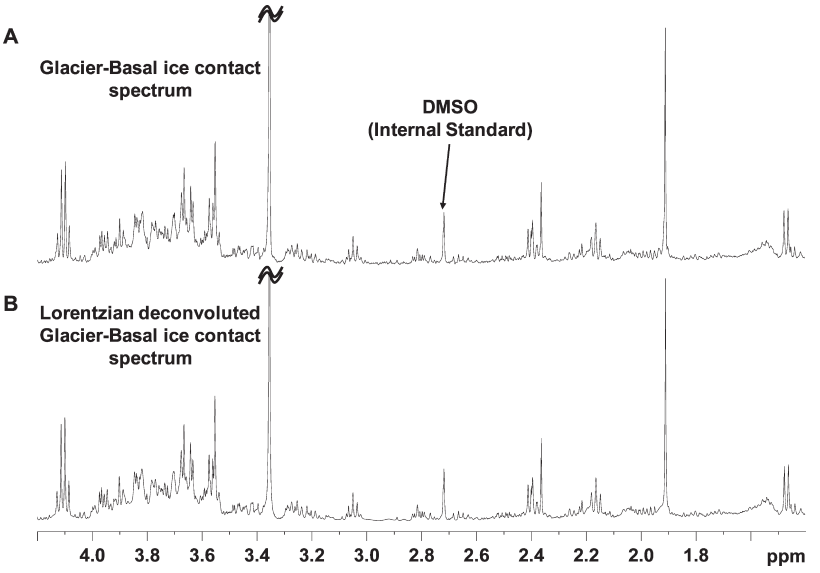

Figure 3. SPR-W5-WATERGATE ^{1}H NMR spectra of the glacial-basal ice contact sample, (A) Magnified spectrum containing 3.4×10^{-10} mol of the DMSO internal standard (2.72 ppm). (B) Example magnified Lorentzian deconvoluted spectrum used to measure peak areas and used in subsequent quantification calculations. The similarity between the two spectra indicates that the Lorentzian deconvolution method does not considerably alter the DOM NMR spectrum.

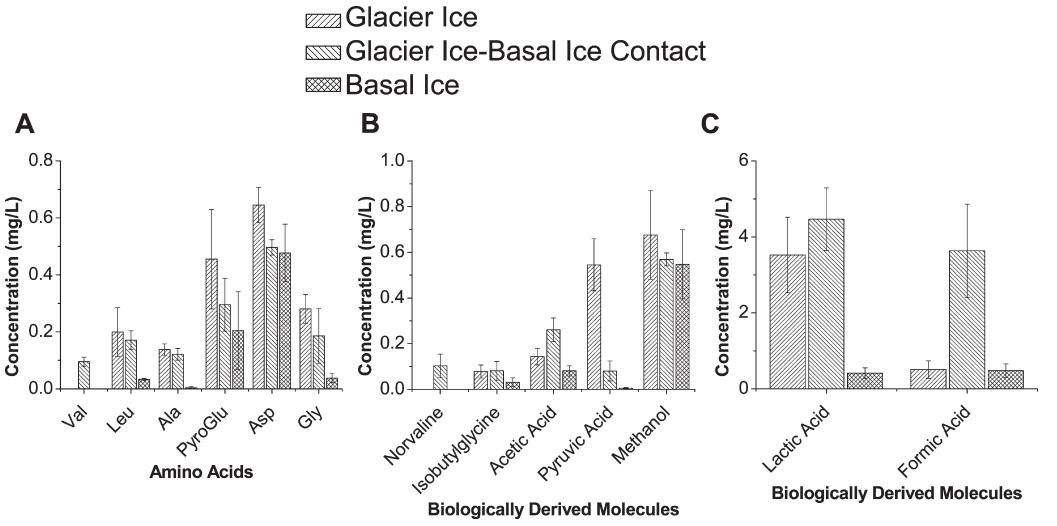

Figure 4. Calculated concentrations (mg/L) of the DOM constituents that contained a fully resolved ^{1}H resonance, (A) amino acids, (B) biologically derived molecules, (C) biologically derived lactic and formic acid. Error bars indicate standard deviation of calculated concentrations from triplicate samples.

glacial DOM is mainly composed of compounds arising from *in situ* microbial metabolism in both glacier and basal ice, supporting its recent fluorescence spectroscopic classification as being predominantly proteinaceous in character.[17,18] The detection of other biomolecules within the ice that are associated with bacterial energy cycles (lactic and pyruvic acid) and metabolism byproduct (such as MeOH and small organic acids) further supports this hypothesis.[44] An additional microbial contribution to basal ice may come from glacially overridden soil organic matter, which is dominated by microbial constituents in the McMurdo Dry Valleys.[45,46]

Variations in DOM composition and abundance have been shown experimentally to stimulate microbial activity; increased inputs of both biologically labile (fast cycling) and aquatic humic substance DOM resulted in the preferential rapid mineralization of labile constituents and net losses of free amino acids.[39,47,48] The application of SPR-W5-WATERGATE ^{1}H NMR spectroscopy has allowed the identification of many of the molecular constituents present in the DOM in glacial ice, providing a basis for predictions of its chemical fate and cycling. For example, if this DOM (which is composed predominantly of amino and small organic acids) is released from glaciers as a result of increased melt and runoff, it could alter the carbon cycling and biogeochemistry of surrounding aquatic ecosystems by stimulating microbial activity through increased inputs of these biologically labile constituents therefore enhancing storage of dissolved organic nitrogen as aquatic microbial biomass.[47,49] This molecular-level approach agrees with the recent findings by Hood et al.[5]

that accelerated melting of glacial ice could generate an increasingly important source of DOM nutrients in glacially fed watersheds. SPR-W5-WATERGATE ^{1}H NMR detects any compound containing protons and thus can be considered a non-selective method considering that the majority of compounds known to be present in the aquatic environment contain at least one proton. However, it should be noted that the glacier ice samples analyzed in this study have relatively high DOC concentrations. Thus, the extension of the SPR-W5-WATERGATE ^{1}H NMR to other samples that are lower in DOC (<1 mg/L) may require the use cryogenically cooled NMR probes that offer improved sensitivity or probes of much larger diameter (10 mm or 15 mm) that permit more sample to be analyzed. Nonetheless, this initial study demonstrates that glacier ice samples can be analyzed with little sample preparation using a static, room temperature NMR probe, and the wealth of qualitative and quantitative information that can be garnered.

■ ASSOCIATED CONTENT

Supporting Information. An example ^{1}H NMR quantification calculation and figures illustrating the identification of the major glacial DOM constituents and method blank are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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