Subsurface ice as a microbial habitat

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ABSTRACT

We determine the physicochemical habitat for microorganisms in subsurface terrestrial ice by quantitatively constraining the partitioning of bacteria and fluorescent beads (1–10 μm) between the solid ice crystals and the water-filled veins and boundaries around individual ice crystals. We demonstrate experimentally that the partitioning of spherical particles within subsurface ice depends strongly on size but is largely independent of source particle concentration. Although bacteria are shown consistently to partition to the veins, larger particles, which would include eukaryotic cells, become trapped in the crystals with little potential for continued metabolism. We also calculate the expected concentrations of soluble impurities in the veins for typical bulk concentrations found in natural ice. These calculations and scanning electron microscope observations demonstrate a concentrated chemical environment (3.5 *M* total ions at −10 °C) in the veins, where bacteria were found to reside, with a mixture of impurities that could sustain metabolism. Our calculations show that typical bacterial cells in glacial ice would fit within the narrow veins, which are a few micrometers across. These calculations are confirmed by microscopic images of spherical, 1.9-μm-diameter, fluorescent beads and stained bacteria in subsurface veins. Typical bacterial concentrations in clean ice (10^2–10^3 cells/mL) would result in concentrations of 10^6–10^8 cells/mL of vein fluid, but occupy only a small fraction of the total available vein volume (<0.2%). Hence, bacterial populations are not limited by vein volume, with the bulk of the vein being unoccupied and available to supply energy sources and nutrients.

Keywords: ice, glacier, water veins, bacteria, eukaryote, bacterial habitat.

INTRODUCTION

Until recently, ice on Earth was thought to be devoid of life due to a combination of low temperatures, absence of liquid water, dilute chemical environment, and, in the subsurface, lack of light and high pressures. Detection of significant bacterial populations (10^1 to 10^7 cells/mL) in glaciers (Sharp et al., 1999; Skidmore et al., 2000) and ice sheets (Karl et al., 1999; Priscu et al., 1999; Bulat et al., 2004) has, therefore, led to speculation about whether bacteria are inactive and deep-frozen, or active within specific physicochemical microenvironments.

The physical structure of polycrystalline ice in glaciers and ice sheets contains a chemically concentrated water phase that takes the form of an interconnected network of highly concentrated water-filled veins (Fig. 1) and films around the crystals (Nye and Frank, 1973; Mader, 1992a; Baker et al., 2003; Barnes et al., 2003). It has been hypothesized that this vein network might provide an aqueous environment and sufficient nutrients for active bacterial metabolism (Price, 2000). Although the general structure of the vein system is known, its detailed physicochemical characteristics and the partitioning of bacteria into it has not been quantified.

We have conducted a series of experiments using a model laboratory system of subsurface ice (i.e., ice where the vein system is well developed) in which the partitioning of bacteria and various sizes of fluorescent beads can be observed.. The advantage of using a model system is that partitioning into the veins can be directly determined, rather than inferred, as both source and end-member conditions are observable. We consider the laboratory ice to be a good model for natural ice produced in situations that involve melting and refreezing (e.g., firnification, basal and superimposed ice). However, even in regions where there is no surface melting (e.g., the central polar ice sheets), a vein system exists that carries the bulk of the soluble impurities and often also the insoluble particles (Price, 2000; Baker et al., 2003; Barnes et al., 2003; Barnes and Wolff, 2004). The results of the laboratory experiments are coupled with determination of the vein conditions (size and chemistry) based on known vein physics, chemical partitioning criteria, and typical impurity loads in glacial ice, plus bacterial counts from a Spitsbergen glacier. Thus, we here constrain quantitatively the nature of the physicochemical habitat of bacteria in the subsurface of polycrystalline ice, and hence the likelihood of microbial viability in ice.

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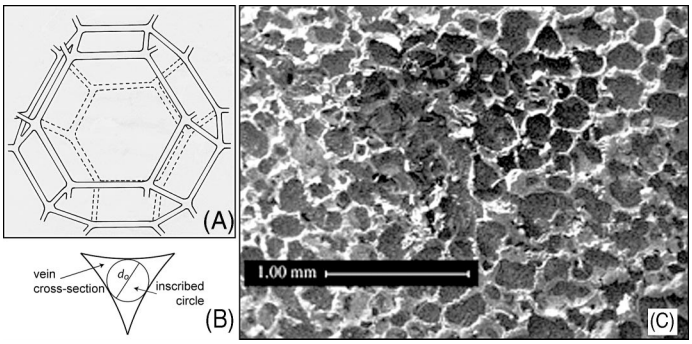

Figure 1. A: Water-filled veins extend along the lines where three crystals meet, forming an interconnected network. (Reproduced from Price, 2000.) B: Veins form approximately equilateral triangles in cross section, with bowed-in faces (Mader, 1992a). Vein cross-sectional size is given by d_o. C: Concentrating effect of veins is illustrated by this SEM image. Sample was cut from center of an ice cube made by freezing 1 *M* NaCl solution at −20 °C. NaCl skeleton remaining after sublimation of the crystal is clearly visible. (Reproduced from Barnes et al., 2003.)

METHODS

Partitioning Experiments

Polycrystalline ice samples were formed in 30 mm glass rings attached to microscope slides from 18.2 Ω deionized water containing either fluorescent (excitation 469 nm, emission 509 nm) polystyrene beads (diameter 1–10 μm) or cells (end of log phase) of *Clostridium vincentii* (a psychrophilic, anaerobic bacterium isolated from a pond on the McMurdo Ice Shelf [Mountfort et al., 1997]) stained with 0.06 g/mL Acridine Orange (5 min). Samples were formed on an aluminum block, chilled by the throughput of 50% antifreeze at −3.5 °C and with freezing rates between about 0.4 and 0.6 μL/s. Continuous stirring prevented bubbles of gas collecting at the freezing front. The Acridine Orange–stained cells were washed prior to freezing so that no free stain was incorporated into the ice. Extensive cell lysis during freezing did not occur as (1) in subsequently melted ice samples, all bacterial cells were intact with no sign of fluorescently stained cell debris, and (2) unstained cells of *Clostridium vincentii* put through the same freezing and thawing conditions were viable. Samples were viewed on a microscope cold stage (Linkham Scientific Instruments, UK) using a long-working-distance (12 mm × 40) objective and concurrent transmitted and epifluorescent light (Zeiss Axioskop microscope with a 50 W mercury vapor lamp and wide-band interference filter set for blue excitation) to determine the distribution of fluorescent particles within the vein network and ice grains. The long working distance allowed us to scan all the way through the samples, thereby capturing the full three-dimensionality of the vein system.

Bacterial Counts for the Glacier Midre Lovénbreen, Spitsbergen

Snow pits were dug at the accumulation and ablation zone. Samples were taken with a sterilized (70% ethanol) spade and placed in presterilized bags. They were melted at base camp and fixed with sterile filtered (0.2 μm) formaldehyde to a final concentration of 2.5%. Ten milliliters of the fixed sample was stained for three minutes with 50 μL of 1 gml^{-1} Acridine Orange solution made up in 2% acetic acid in distilled water. The stained sample was filtered through a black 0.2 μm polycarbonate filter, and the number of fluorescent bacteria was determined microscopically (Cragg and Parkes, 1993).

Vein Chemistry Calculations

A chemical composition for typical glacial ice was established from values given in the literature (Table 1). The bulk impurity concentration of those compounds that partition to the veins was then determined based on the fact that ions that are large and incompatible with bonding in the lattice are expected to be partitioned mainly to the veins and films; even for polar ice, this has been observed in several studies (e.g., Barnes and Wolff, 2004; Baker et al., 2003). Concentrations for these compounds are given in Table 1. It is known that some ions, such as Cl^- and NH_4^+, can be incorporated substitutionally into the crystal lattice (e.g., Petrenko and Whitworth, 1999, their section 5.4). These ions will be uniformly distributed at concentrations (typically 1–10 μ*M*) that are negligible compared to the concentrations given in Table 1. It is unclear how dissolved organic carbon (DOC) partitions, and so it was not included in the calculations. However, as DOC likely contains some large-molecular-weight compounds and is of small particle size (0.2–0.45 μm), some components should partition into the veins.

TABLE 1. CHEMISTRY OF VEIN WATER

	Typical Bulk Concentration* $\left(\frac{\times 10^{-6}\ \text{moles of ions}}{\text{liter of bulk ice}}\right)$		Vein Concentration $\left(\frac{\text{moles of ions}}{\text{liter of vein}}\right)$	
		References*	−1 °C	−10 °C
Compound§				
HCOOH	2	a,b	0.069	0.693
$H_2C_2O_4$	0.05	c	0.002	0.017
CH_3COOH	4	b	0.139	1.387
$HSO_4^- + X^+$	2	d,e,f	0.069	0.693
$NO_3^- + X^+$	6	a,d	0.208	2.080
$CH_3SO_3^- + X^+$	0.2	a,f	0.007	0.069
Totals				
Ionic Concentration†	14.25	N.A.#	0.494	4.94
Molarity†	10.15μM	N.A.#	0.352M	3.52M

Note: The total water content (volume of water per volume of bulk ice) is given by the ratio of the bulk concentration to the vein concentration. To calculate the water content in the *veins*, the loss of material to the grain boundary films must be accounted for; at −1 °C reduce the total water content by 2%, 1% and 0.1% and at −10 °C by 20%, 6% and 1% for crystal sizes of 1.5 mm, 5 mm, and 50 mm respectively. The crystal shape used is a semiregular truncated octahedron. This commonly used approximation was first proposed by Frank (1968) and Nye and Frank (1973) because it is space-filling such that grain edges are *triple junctions* (lines where 3 grains meet) as in ice polycrystals.

*Typical bulk concentrations are median values of ranges obtained from the literature. a: Ginot et al., 2002; b: Legrand et al., 2003; c: Kang et al., 2001; d: Wadham, 1997; e: Doscher et al., 1995; f: Isaksson et al., 2001.

§Compounds that dissociate are identified by their anion and a generalized cation (X^+).

†The ionic concentration is the total moles of *ions* per liter of bulk ice or vein. The molarity is the moles of *molecules* per liter of bulk ice or vein. In converting from ionic concentration to molarity, careful attention must be paid to the dissociation of the particular compound in question.

#N.A. = not applicable.

Vein Size Calculations

The vein size can be calculated from known equations for the vein geometry (e.g., Nye and Frank, 1973; Mader, 1992a, 1992b), given the prevailing temperature (which fixes the ionic concentration in the veins and films), the bulk ionic concentration of impurities that partition to the veins (Table 1), and the crystal size and shape. In converting from bulk concentration (discussed in the previous paragraph) to bulk ionic concentration, we assume that sulfuric, nitric, and methane sulfonic acids dissociate into two ions per molecule, whereas the organic acids (formic, oxalic, acetic) do not dissociate due to the low pH (we expect a low pH, as much of the cation X^+ is acidity) and so are present in molecular form. The crystal size and shape fixes the total length of veins and area of films for a given crystal size. Impurities are partitioned first into the films (maximum 10^{18} molecules/m^2 at −10 °C; 10^{17} molecules/m^2 at −1 °C) and then into the veins (Barnes et al., 2003; Baker et al., 2003; Barnes and Wolff, 2004).

RESULTS

The results of the partitioning experiments are shown in Figures 2 and 3. The proportion of beads incorporated into the bulk ice in-

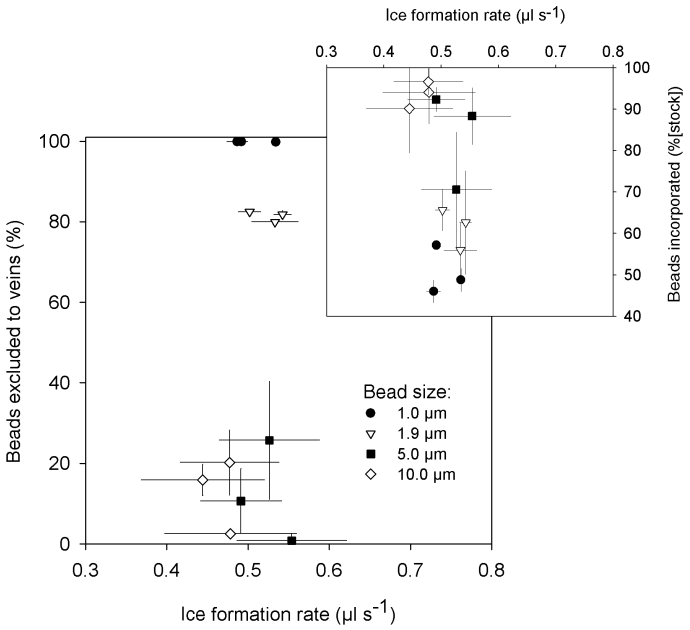

Figure 2. Main graph shows beads excluded to veins as a percentage of total number of beads incorporated into bulk ice. Inset graph shows percentage incorporation of beads into bulk ice. Ice formation rates were calculated from measurements of the time taken for known volumes of water to freeze completely.

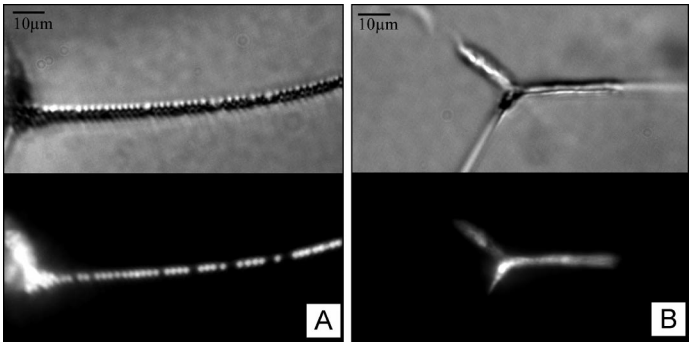

Figure 3. Light (top) and fluorescence (bottom) micrographs of 1.9 µm fluorescent beads showing the beads lined up along the water vein and into node (A), and of *Clostridium vincentii* (Mountfort et al., 1997) stained with Acridine Orange localized along a vein and node (B).

creased with bead size, as is consistent with theory (Rempel and Worster, 2001). Fewer than 60% of small beads (1 and 1.9 µm) were incorporated, compared with ~90% of large beads (5 and 10 µm) (Fig. 2 inset). These proportions were independent of bead concentration over four orders of magnitude. The proportion of beads excluded to the veins was also strongly dependent on size, with only ~20% of large beads excluded compared to more than 80% of small beads (Fig. 2). Almost all the 1 µm beads incorporated into the bulk ice were excluded to the vein system (Fig. 3A). Although there are few estimates of the size of bacteria in clean ice, data suggest relatively small sizes (e.g., Sheridan et al. [2003] find that the majority are ≤1 µm, and Karl et al. [1999] find ~50% coccoids, 0.1–0.4 µm, and ~50% thin rods and vibrios, 0.5–3 µm), which suggests that most bacteria will similarly be excluded into the vein network. This was confirmed in the experiments using fluorescently labeled *Clostridium vincentii* cells where bacteria were only detected in veins and nodes and never in the ice crystals themselves (Fig. 3B). The bacterial dimensions (width 0.6 µm, length 2.5–5 µm) suggest that exclusion to veins is controlled by the smallest dimension.

Total bacterial counts in the samples from the glacier Midre Lovénbreen, Spitsbergen, ranged from 10^2 to 10^3 cells/mL of bulk ice and are similar to other values for subsurface ice, even the deep accreted ice from Lake Vostok (Karl et al., 1999; Priscu et al., 1999). Hence, the Midre Lovénbreen counts are representative for terrestrial glacial ice and can be used to calculate likely bacterial concentrations in ice veins. The calculations of vein chemistry (Table 1) show that the vein solution is concentrated by a factor of 10^4 to 10^5 (for −1 to −10 °C, 10 µ*M* bulk concentration) compared to typical bulk ice. As bacteria partition to the veins (Figs. 2 and 3), the concentration factor is the same as for chemicals, giving 10^6–10^8 cells/mL of vein fluid. If these were 1–2-µm-diameter spherical bacteria, they would occupy a negligible percentage of the vein volume (<0.2%). In addition, the vein cross-sectional size (Fig. 1B) is d_o = 2.4–4.5 µm. Thus, spheres with diameter 1.9 µm would to be forced to line up along the veins, which is exactly what occurs in our experiments (Fig. 3A). At temperatures as low as even −40 °C, we calculate that, for 10 µ*M* bulk concentration, 1-µm-diameter spherical bacteria will still reside in a liquid environment as d_o = 1.4 µm and 10^3 cells/mL of bulk ice occupy <0.1% of the vein volume. Hence, individual bacterial cells are not limited by vein volume, with the bulk of the vein unoccupied and available to supply energy sources and nutrients.

DISCUSSION

Total molarities in the veins and films are in the range typically used in rich laboratory media (0.3–3 *M*, Table 1), and hence the vein habitat is not a dilute environment. It has been shown that bacteria in sea ice can be metabolically active in high-ionic-strength solutions (Junge et al., 2004). Formate, acetate, oxalate, methanesulfonate, and DOC in veins can all be used as energy and carbon sources by a variety of bacteria. For example, *Proteobacteria* are present in glacier ice (Miteva et al., 2004) and some members of this phylum can utilize methanesulfonate for energy, carbon, and sulfur (Holmes et al., 1997). With the exception of methanesulfonate (Kelly and Murrell, 1999), these vein components can be utilized even under the anaerobic conditions that develop in some glaciers (Wadham et al., 2004). Sulfate, nitrate, and any humic acids in the DOC (Lovley et al., 1996) can be used in anaerobic respiration, while nitrate and ammonia could be nitrogen sources and sulfate a sulfur source. Hence, a range of concentrated chemicals is available to facilitate bacterial metabolism. As small particles are also excluded to the vein system, these could provide additional substrates and nutrients such as phosphate. Bacterial colonization of particles in veins for energy, etc., might explain why, in both sea and glacial ice, bacterial populations were significantly correlated with particles (Sharp et al., 1999; Sheridan et al., 2003; Junge et al., 2004). Some bacteria might also be carried into the veins on small particles.

Partitioning of beads and bacteria (Figs. 2 and 3) demonstrate that bacteria less than ~2 µm in diameter would predominantly be excluded into the vein system, whereas larger eukaryotic cells (~10 µm), such as snow algae, would be trapped within ice crystals. Hence, the local physicochemical environment would be very different for the two cell types, with much greater potential for continued metabolism for the bacteria in the highly concentrated water veins (Table 1). This finding is consistent with reports that low-salinity sea ice had no habitable space for sea algae (Granskog et al., 2003), yet near-surface sea-ice bacteria were associated with ice-crystal boundaries and active at −20 °C (Junge et al., 2004).

It has recently been suggested that there is no temperature minimum for metabolism down to at least −40 °C, and hence that bacteria in much terrestrial ice should still be active (Price and Sowers, 2004). However, the low temperatures will slow metabolism, possibly enabling substrates, etc., to last over long time scales. Consequently, metabolic products can gradually accumulate potentially changing concen-

trations of CO_2, N_2O, SO_4^{2-}, CH_4 and other compounds, with important implications for paleoatmosphere studies using ice chemistry (Campen et al., 2003).

The Midre Lovénbreen bacterial counts are representative of ''clean'' ice and hence glaciers generally. With a global ice volume of order 10^7 km^3, this represents a likely global total of 10^{24}–10^{25} bacterial cells in ice, with the majority in the liquid, highly concentrated vein network. Although this is small compared with bacterial numbers in other present-day environments (Whitman et al., 1998), it clearly demonstrates that ice is a significant habitat for bacteria, which would have been even more important during colder periods, e.g., Snowball Earth (Hyde et al., 2000).

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