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Research Paper

Hydrogeologic Controls on Episodic H_2 Release from Precambrian Fractured Rocks—Energy for Deep Subsurface Life on Earth and Mars

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ABSTRACT

Dissolved H_2 concentrations up to the m*M* range and H_2 levels up to 9–58% by volume in the free gas phase are reported for groundwaters at sites in the Precambrian shields of Canada and Finland. Along with previously reported dissolved H_2 concentrations up to 7.4 m*M* for groundwaters from the Witwatersrand Basin, South Africa, these findings indicate that deep Precambrian Shield fracture waters contain some of the highest levels of dissolved H_2 ever reported and represent a potentially important energy-rich environment for subsurface microbial life. The δ^2H isotope signatures of H_2 gas from Canada, Finland, and South Africa are consistent with a range of H_2-producing water-rock reactions, depending on the geologic setting, which include both serpentinization and radiolysis. In Canada and Finland, several of the sites are in Archean greenstone belts characterized by ultramafic rocks that have undergone serpentinization and may be ancient analogues for serpentinite-hosted gases recently reported at the Lost City Hydrothermal Field and other hydrothermal seafloor deposits. The hydrogeologically isolated nature of these fracture-controlled groundwater systems provides a mechanism whereby the products of water-rock interaction accumulate over geologic timescales, which produces correlations between high H_2 levels, abiogenic hydrocarbon signatures, and the high salinities and highly altered δ^{18}O and δ^2H values of these groundwaters. A conceptual model is presented that demonstrates how periodic opening of fractures and resultant mixing control the distribution and supply of H_2 and support a microbial community of H_2-utilizing sulfate reducers and methanogens. Key Words: Hydrogen—Methane—Gases—Hydrocarbons—Groundwater—Continental crust—Fractures—Chemoautotrophic—Serpentinization—Radiolysis. Astrobiology 7, 971–986.

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INTRODUCTION

In the recent literature, there has been significant interest in chemoautotrophic microorganisms and their role in the deep terrestrial biosphere, in Earth's earliest microbial ecosystems, and as potential analogues for life on other planets. Serpentinization of ultramafic rocks and alteration of basaltic ocean floor have been invoked as key mechanisms by which geochemical processes of water-rock interaction may provide energy and reducing power for chemoautotrophic microbial communities on the seafloor (Bach and Edwards, 2003; Bach *et al*., 2004; Charlou *et al*., 2002; Jannasch, 1985; Kelley *et al*., 2005; Nealson *et al*., 2005; Schulte *et al*., 2006; Takai *et al*., 2004). Several models have been developed to examine the significance of similar H_2- and CH_4-generating processes for chemoautotrophic life on early Earth and, potentially, Mars (Boston *et al*., 1992; Lyons *et al*., 2005; Oze and Sharma, 2005; Sleep *et al*., 2004). Though a significant number of studies have focused on ocean-floor processes, investigations into H_2- and CH_4-generating processes in deep groundwaters in continental settings have been somewhat infrequent. Chapelle *et al*. (2002) and Spear *et al*. (2005) reported H_2-utilizing chemoautotrophic microbial communities in volcanic hot springs, while Stevens and McKinley (1995) suggested that H_2 autotrophic microbial ecosystems might be important at 1.3 km depth in continental flood basalts. In saline groundwaters in fractured crystalline rock at 2–3 km depth in the Witwatersrand Basin of South Africa, Lin *et al*. (2006b) and Sherwood Lollar *et al*. (2006) showed microbiological and molecular evidence, and geochemical and isotopic signatures, that were consistent with *in situ* microbial communities linking H_2 consumption to sulfate reduction and methanogenesis. Building on previous studies of sites in Canada, Finland, and South Africa, the current paper shows that, worldwide, Precambrian Shield fracture waters represent potential energy-rich environments for subsurface life, based on some of the highest levels of dissolved H_2 ever measured in groundwaters. The isotopic signature of the H_2 from sites on the Canadian and Fennoscandian shields, as well as for the Witwatersrand Basin, South Africa, are presented, and possible origins for H_2 in these tectonically stable ancient continental shield terrains are discussed. A geologic and hydrogeologic conceptual model is presented to account for the high levels of H_2 observed in deep fracture waters and the absence of H_2 in other parts of the hydrogeologic network. The relationship of the differential distribution of this energy source to microbial communities is discussed, as are implications with regard to fracture-controlled episodic release of substrates such as H_2, microbial activity in the terrestrial deep subsurface, and the search for extinct or extant life on Mars.

MATERIALS AND METHODS

Sampling methods

In Canada and South Africa, gas phase and fracture water samples were collected from underground boreholes at the borehole collar after the method of Sherwood Lollar *et al*. (2002) and Ward *et al*. (2004). A packer was placed into the opening of the borehole and sealed to the inner rock walls below water level to seal the borehole from the mine air and minimize air contamination. Gas and water were allowed to flow through the apparatus long enough to displace any air remaining in the borehole or the apparatus before sampling. Plastic tubing was attached to the end of the packer, and the flow of gas or water from the borehole was directed into an inverted graduated funnel. Gases collected in the inverted funnel were transferred directly into evacuated vials through a needle that was attached to the top of the funnel. The gas sampling vials were pre-evacuated 130 ml borosilicate vials sealed with butyl blue rubber stoppers prepared after the method of Oremland and Des Marais (1983). Vials were pre-fixed with 50 μl of a saturated $HgCl_2$ solution to kill any microbes contained in the sample so that microbial activity post-sampling would not alter the gas composition and isotopic signatures. Previous studies that have compared the isotopic values of gases taken at the borehole collars to values determined for gases in solution at depth in the same boreholes showed that exsolution of the hydrocarbon gases from solution does not alter their isotopic signatures (Sherwood Lollar *et al*., 1993a, 1994, 1993b).

In Finland, samples were also collected from uncased boreholes, but in this case the boreholes were at land surface rather than underground. Samples were collected with the use of downhole sampling probes designed to collect pressurized samples of dissolved gas and groundwater to depths of 1000 m below surface (Sherwood Lollar *et al*., 1993a, 1994). Probes (fitted with soft copper tube sampling chambers) were lowered to the sampling depth and allowed to equilibrate. Once filled, sample chambers were maintained at sampling depth pressure by gas-tight valves as samples were returned to surface. At surface, the copper tubes were clamped off in accordance with the technique traditionally used for noble gas analysis, which sealed the pressurized fluid and dissolved gases in the sampling tubes. Gas phases and associated fluid were then quantitatively extracted in the laboratory by vacuum degassing techniques to determine moles H_2 per liter of water (Sherwood Lollar *et al*., 1993a, 1994). This technique of gas sampling and storage is well established in noble gas geochemistry since this method ensures no loss of light gases such as He, Ne and H_2.

Compositional analysis

Compositional analyses of gas phase samples were performed on a Varian 3400 equipped with a flame ionization detector to determine concentrations of CH_4, C_2H_6, C_3H_8, and C_4H_{10}. The hydrocarbons were separated on a J&W Scientific GS-Q column (30 m $\times$ 0.32 mm ID) with a helium gas flow and the following temperature program: initial temperature of 60ºC, hold for 2.5 minutes, increase to 120°C at 5ºC/min. Both an HP 5830A and a Varian 3800 GC equipped with a microthermal conductivity detector and Molecular Sieve 5A PLOT columns (25 m $\times$ 0.53 mm ID) were used to determine concentrations of the inorganic gas components (H_2, He, Ar, O_2, CO_2 and N_2). To determine concentrations of H_2, the argon carrier gas flow rate was 2mL/min and the temperature program was initial temperature of 10°C, hold for 10 min, increase to 80°C at 25°C/min, hold for 7 min. All analyses were run in triplicate, and mean values are reported in Table 1. Reproducibility for triplicate analyses was $\pm$5%. Detection limit for H_2 by this method was 0.01% by volume (Table 1).

For the South African samples, dissolved H_2 concentrations were determined by way of a Kappa-5 Reduced Gas Analyzer (RGA 5, Trace Analytical, Sparks, MD) equipped with a Hg vapor detector for H_2 after the method of Lin *et al*. (2005a). Gases were extracted from sample bottles with a gas-tight syringe and diluted to a concentration less than 10 ppm. All analyses were run in triplicate, and mean values are reported. Reproducibility for triplicate anlayses was $\pm$5% relative error, comparable to the GC analyses, but the RGA provided a substantially improved detection limit and could quantify dissolved H_2 levels in samples where H_2 was below the GC detection limit (<0.01% by volume). The measured values were converted to dissolved concentrations in accordance with the procedures of Andrews and Wilson (1987), with the use of the Henry's law constant and the groundwater flow rate to gas flow rate ratio measured in the field. Flow rates measured during sampling varied by <10%. Error propagation for the approach yielded an overall uncertainty of $\pm$20% for the reported values.

Lin *et al*. (2005a) reported dissolved H_2 concentrations for the Witwatersrand Basin samples, as well as much higher diffusion-corrected values of dissolved H_2, assuming a model of degassing of fluid internally within a partially dewatered fracture zone based on atmospheric noble gas measurements, after the method of Lippmann *et al*. (2003). In this paper, however, only the measured H_2 concentrations are considered, as they represent the most conservative estimate of dissolved H_2 levels.

Isotopic analysis

Isotopic analyses were performed at the University of Toronto (Table 2). The δ^2H analysis was performed on a continuous-flow compound-specific hydrogen isotope mass spectrometer, which consists of an HP 6890 gas chromatograph (GC) interfaced with a micropyrolysis furnace (1465°C) in line with a Finnigan MAT Delta$^+$-XL isotope ratio mass spectrometer. Total error incorporating both accuracy and reproducibility is $\pm$5‰ with respect to V-SMOW. Analyses for δ^{13}C values were performed by continuous-flow compound-specific carbon isotope ratio mass spectrometry with a Finnigan MAT 252 mass spectrometer interfaced with a Varian 3400 capillary GC. Hydrocarbons were separated by a Poraplot Q™ column (25 m $\times$ 0.32 mm ID) with the following temperature program: initial temperature of 40°C, hold for 1 min, increase to 190°C at 5°C/min, hold for 5 min. Total error incorporating both accuracy and reproducibility is $\pm$0.5‰ with respect to V-PDB standard.

TABLE 1. CONCENTRATIONS OF DISSOLVED H_2 (IN μM) IN GROUNDWATERS AND IN FREE GAS PHASE (IN VOLUME %) FOR SITES FROM THE WITWATERSRAND BASIN, SOUTH AFRICA, AND THE FENNOSCANDIAN AND CANADIAN SHIELDS

Site	*Sample name*	*Depth (in m)*	*Dissolved H_2 (in μM)*	*Gas phase H_2 (in vol. %)*
Witwatersrand Basin, South Africa				
Beatrix	BE16FW031601	866	0.119	<0.01
Beatrix	BE23FW031301	718	3.70	<0.01
Beatrix	BE24FW032601	768	4.44	<0.01
Beatrix	BE325FW032701	1290	0.016	<0.01
Beatrix	BE16FW031401IDW	866	0.097	<0.01
Evander	EV219FW030901	1474	0.774	<0.01
Evander	EV522FW041801	1694	0.025	<0.01
Evander	EV818FW030601	1950	1.28	<0.01
Evander	EV818BH5-102702	1890	10.2	<0.01
Evander	EV818BH6-102702	1890	0.608	<0.01
Evander	EV818BH6-111502	1890	5.21	<0.01
Kloof	KL1GH	NA	NA	9.15
Kloof	KL443FW050201	3100	0.455	<0.01
Kloof	KL443FW030501	3200	25.0	<0.01
Kloof	KL443FW050801	3200	47.3	<0.01
Kloof	KL739062901	3000	4980	9.25
Driefontein	DR938CH1	2712	NA	<0.05
Driefontein	DR548FW090901	3200	7410	10.3
Driefontein	DR938H3110701	2716	165	0.74
Driefontein	DR938H3071202	2716	12.0	0.32
Driefontein	DR4IPC	945	0.090	<0.05
Mponeng	MP104XC56110902	2825	3714	11.5
Mponeng	MP109FW101701	3000	203	3.30
Fennoscandian Shield				
Juuka	116-1	253	2550	NA
Juuka	116-1	498	131	NA
Juuka	116-1	751	79.3	NA
Juuka	116-1	850	328	NA
Juuka	116-2	254	428	NA
Juuka	116-2	500	2190	NA
Juuka	116-2	746	612	NA
Juuka	116-2	890	217	NA
Juuka	116-3	256	1120	NA
Juuka	116-3	750	307	NA
Juuka	116-3	897	2110	NA
Outokumpu	741	850	2.04	NA
Pori		50	6.54	NA
Pori		151	2.04	NA
Pori		202	1.63	NA
Pori		240	94.8	NA
Pori		351	1040	NA
Ylistaro		102	7.36	NA
Ylistaro		202	6.13	NA
Ylistaro		301	3.27	NA
Ylistaro		407	1380	NA
Ylivieska		249	5.72	NA
Canadian Shield				
Sudbury	CS104577	1333	NA	54.0
Sudbury	CS104547	1333	NA	43.0
Sudbury	CS104546	1333	NA	9.94
Sudbury	CS106701	1333	NA	31.6
Sudbury	CS104577	1333	NA	57.8
Sudbury	CS104590	1100	NA	51.0
Sudbury	CS104880	1100	NA	19.7
Timmins	KC5993	2072	NA	2.03
Timmins	KC5990	2072	NA	0.17

TABLE 1. CONCENTRATIONS OF DISSOLVED H_2 (IN μM) IN GROUNDWATERS AND IN FREE GAS PHASE (IN VOLUME %) FOR SITES FROM THE WITWATERSRAND BASIN, SOUTH AFRICA, AND THE FENNOSCANDIAN AND CANADIAN SHIELDS (CONT'D)

Site	Sample name	*Depth (in m)*	*Dissolved H_2 (in μM)*	*Gas phase H_2 (in vol. %)*
Timmins	KC5991	2072	NA	5.43
Timmins	KC6064	2072	NA	0.83
Timmins	KC6070	2072	NA	6.05
Timmins	KC6079	2072	NA	0.91
Timmins	KC6080	2072	NA	4.78
Timmins	KC6158	2072	NA	0.13
Timmins	KC6159	2072	NA	0.83
Timmins	KC6160	2072	NA	0.94
Timmins	KC6161	2072	NA	0.63
Timmins	KC6276	2072	NA	1.00
Timmins	KC6277	2072	NA	1.81
Timmins	KC6292	2072	NA	1.84
Timmins	KC6297	2072	NA	1.96
Timmins	KC6298	2072	NA	12.7
Timmins	KC6299	2072	NA	1.63
Timmins	KC6300	2072	NA	5.44
Timmins	KC6301	2072	NA	0.24
Timmins	KC6360	2072	NA	0.20
Timmins	KC6429	2072	NA	1.79
Timmins	KC6445	2072	NA	1.03
Timmins	KC6446	2072	NA	1.00
Timmins	KC6447	2072	NA	8.70
Timmins	KC6448	2072	NA	0.40
Timmins	KC6498	2072	NA	0.78
Timmins	KC6499	2072	NA	0.99
Timmins	KC6500	2072	NA	0.71
Timmins	KC6501	2072	NA	0.28
Timmins	KC6502	2072	NA	2.16
Timmins	KC7792	2100	NA	0.34
Timmins	KC8558	2100	NA	1.97
Timmins	KC8428	2100	NA	2.45
Timmins	KC8282	2100	NA	1.30
Timmins	KC8402	2100	NA	2.00
Timmins	KC8539	2100	NA	1.34

South African data from Lin *et al*. (2005a).
NA, not analyzed.

RESULTS

An H_2-rich deep biosphere

Several previous studies that have identified chemoautotrophic microbial ecosystems have noted the H_2-rich nature of the groundwaters in which those communities were found. Compared to the n*M* levels of dissolved H_2 typical of anaerobic sediments (Lovley and Goodwin, 1988), groundwaters at Lidy Hot Springs (Chapelle *et al*., 2002), at Yellowstone hot springs (Spear *et al*., 2005), and in the Columbia River basalt aquifer (Stevens and McKinley, 1995) contain dissolved H_2 concentrations of 0.011–0.015 μM, 0.002–0.325 μM, and 0.02–80 μM respectively (Fig. 1). In contrast, fracture waters in Precambrian Shield rocks of the Witwatersrand Basin of South Africa and the Canadian and Fennoscandian shields have some of the highest levels of dissolved H_2 ever measured in groundwaters (Table 1). These values are of the same order of magnitude as H_2 concentrations of 12–16 m*M* reported for ultramafic oceanic vent fluids, such as Rainbow and Logatchev (Charlou *et al*., 2002), and the Lost City Hydrothermal Field (LCHF) (Kelley *et al*., 2005). Unlike these spreading centers, where gas-fluid interactions are dominated by high-temperature chemical (and even isotopic) equilibration (Kelley, 1996; Kelley and Früh-Green, 1999; Welhan and Craig, 1979; Welhan and Craig, 1983), groundwaters in the Precambrian cratons have

TABLE 2. ISOTOPIC VALUES (δ^2H) FOR H_2 IN ‰ FOR FREE GAS PHASE SAMPLES FROM THE WITWATERSRAND BASIN, SOUTH AFRICA, AND THE FENNOSCANDIAN AND CANADIAN SHIELDS

Site	*Sample name*	*Depth (in m)*	*δ^2H H_2 (in ‰)*
Witwatersrand Basin, South Africa			
Kloof	KL1GH	NA	−684
Kloof	KL739062901	3000	−682
Driefontein	DR548FW090901	3200	−706
Driefontein	DR938H3110701	2716	−685
Driefontein	DRGS110198	945	−700
Driefontein	DR638GS110198	2000	−685
Mponeng	MP104XC56110902	2825	−684
Fennoscandian Shield			
Juuka	116-1	0	−619*
Juuka	116-1	254	−649*
Pori		350	−659*
Canadian Shield			
Sudbury	CCS59501	1333	−637*
Sudbury	CCS104577	1333	−719
Sudbury	CCS104547	1333	−725
Sudbury	CCS104546	1333	−738
Sudbury	CCS104577	1333	−730
Sudbury	CCS104590	1100	−728
Sudbury	CCS104880	1100	−732
Timmins	KC8558	2100	−732
Timmins	KC8428	2100	−725
Timmins	KC8282	2100	−737
Timmins	KC8402	2100	−726
Timmins	KC8539	2100	−734

*Sampled in 1988 (Sherwood Lollar *et al.*, 1993b).
NA, not available.

not experienced such high temperatures for millennia and are dominated by slower processes of low-temperature interaction. The South African groundwaters typically have H_2 concentrations in the n*M* to m*M* level, with measured concentrations in the deepest fracture waters in South Africa increasing to values as high as 7.4 m*M* (Fig. 1). In the Fennoscandian Shield, H_2 concentrations range from 0.1–2.5 m*M* at all sites (Fig. 1). For sites on the Canadian Shield, H_2 concentrations were only characterized for the free gas phase degassing from flowing groundwaters (Table 1). Without routine measurement of the corresponding groundwater flow rates from the boreholes, the gas flow rates could not be expressed in terms of dissolved H_2 concentrations. Hence, for the Canadian Shield sites the H_2 levels are reported only as % by volume of the free gas phase. A comparison between the dissolved gas measurements and free gas phase values for all sites for which both measurements are available can be seen in Table 1. Table 1 also shows that H_2 levels can be as high as 9–58% of the total free gas phase.

H_2 isotopic signatures

The δ^2H isotopic values measured in the Precambrian Shield fracture waters in Canada, Finland, and the Witwatersrand Basin occupy a relatively narrow range from −619 to −738‰ (Table 2; Fig. 2). This range is very similar to that reported in previous studies for H_2 gas from 2 continental gas wells in Kansas (Coveney *et al.*, 1987) and from ophiolite-hosted gas seeps in Oman (Fritz *et al.*, 1992; Neal and Stanger, 1983) (Fig. 2). Isotopic values originally reported for H_2 from gas seeps in the Zambales ophiolite sequence in the Philippines were significantly more enriched (−581‰, and −599‰) (Abrajano *et al.*, 1990). Based on a 2005 expedition to, and re-sampling of, the Los Fuegos Eternos site and a new reduced-gas occurrence in the Philippines, however, measured values reported here for the first

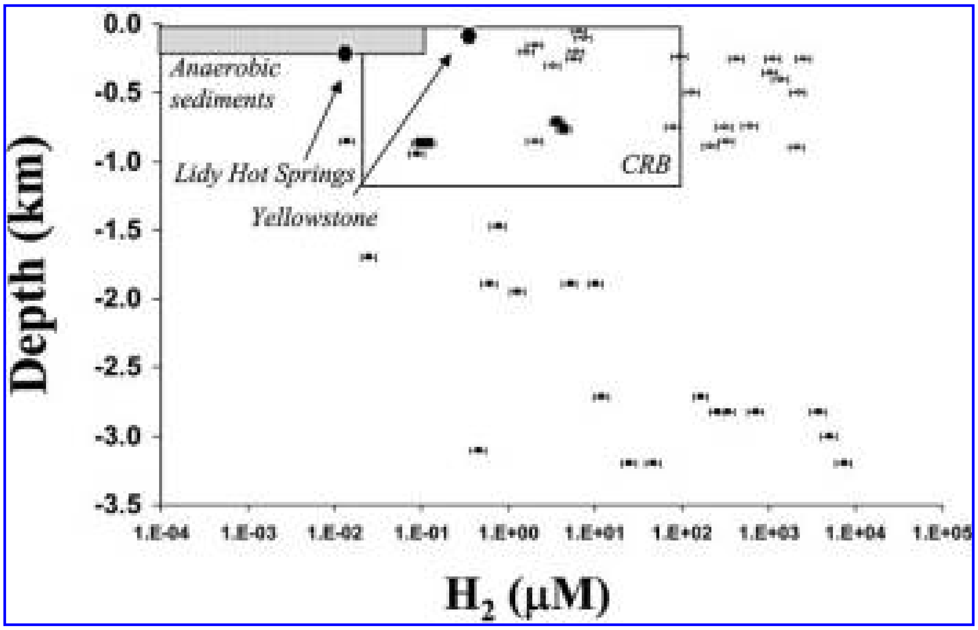

FIG. 1. Dissolved H_2 concentrations (in μM) for groundwaters from the Fennoscandian Shield (open circles) and for the Witwatersrand Basin, South Africa (closed squares). For comparison, dissolved H_2 concentrations for porewaters and groundwaters from anaerobic sediments (Lovley and Goodwin, 1988); for the Columbia River basalt (CRB) aquifers (Stevens and McKinley, 1995); and maximum values reported for Lidy Hot Springs (Chapelle *et al*., 2002) and Yellowstone hot springs (Spear *et al*., 2005) are shown. South African data are from Lin *et al*. (2005a).

time (Los Fuegos: −679‰, −707‰; new site: −632‰, −648‰, −650‰) for δ^2H of H_2 more closely conform with the other values in Fig. 2. Although H_2 in seafloor hydrothermal systems, such as the LCHF range to more-enriched δ^2H values due to isotopic equilibration of H_2 with the higher temperature vent fluids in these systems (Proskurowski *et al*., 2006), the data from this study and from the literature indicate that H_2 in groundwaters typically has δ^2H values that fall in the range of −600 to −800‰, with the majority falling in a relatively narrow range between −675 and −750‰ (Fig. 2).

Lin *et al*. (2005b) demonstrated that, while the δ^2H isotopic values for H_2 produced in laboratory experiments by radiolytic decomposition of water were in the range of −348 to −539‰, the isotopic composition of H_2 produced by radiolysis in the natural environment would be much more ^2H depleted due to isotopic re-equilibration with *in situ* groundwaters whose residence time exceeded 10^3 to 10^5 years. Figure 3 demonstrates that the range of δ^2H values for H_2 in relatively low-temperature groundwaters can be predicted, assuming isotopic equilibration between water and H_2. For a range of temperatures from 0 to 60°C , and typical δ^2H_{H2O} values for groundwaters of +10‰ to −120‰ (Clark and Fritz, 1997), δ^2H values for H_2 in the system are likely to be constrained to the range −680 to −800‰ if the residence time for H_2 in groundwaters is on the order of thousands of years or more. Regardless of the original δ^2H value of the H_2 produced, isotopic re-equilibration is likely to overprint the primary isotopic value of H_2 and result in very depleted δ^2H values for the gas due to relatively rapid H_2-H_2O isotopic exchange on geologic timescales.

DISCUSSION

Origin of H_2

An important corollary to the phenomenon illustrated in Fig. 3 is that, while δ^2H_{H2} values may provide information about the temperature of equilibration between H_2 and water in most groundwater settings, the values provide less reliable information about the origin of the H_2. In practice, determining the origin of the H_2 in a given system is largely dependent on assessing the geochemical and mineralogical setting and thermodynamic potential for various possible H_2-

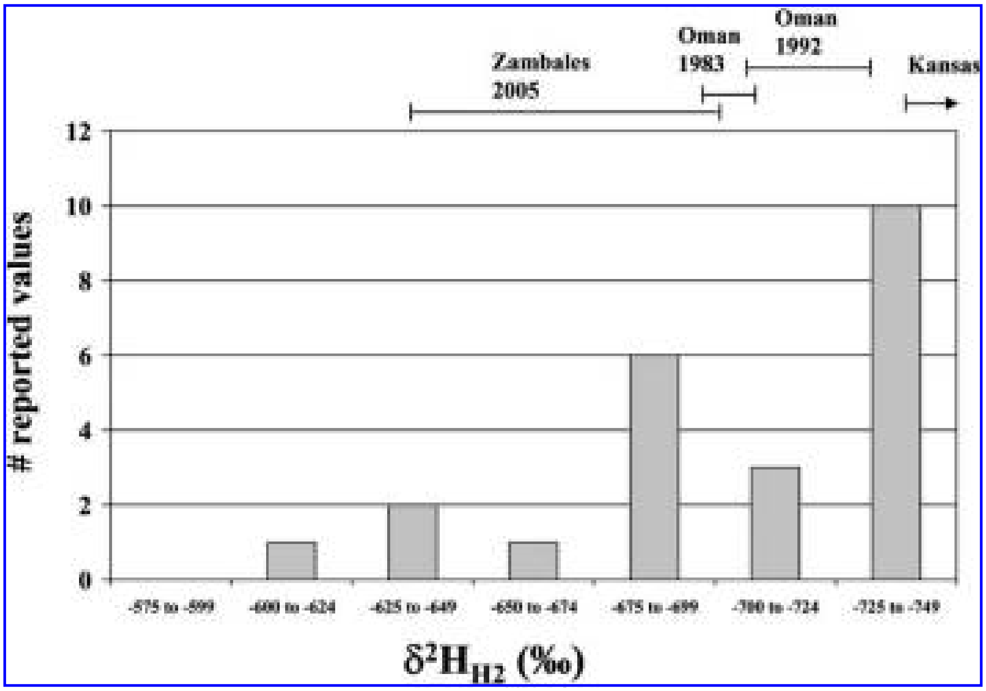

FIG. 2. Distribution of δ^2H values for H_2 from sites in Precambrian Shield rocks of Finland, Canada and South Africa. The most ^2H-enriched values (−619‰, −637‰, −649‰, −659‰) were all from sites sampled in 1988 and were analyzed by off-line sample preparation techniques with a poorer reproducibility (±20‰) compared to current analytical capabilities by continuous-flow compound-specific isotope analysis (±5‰). Hence the more-enriched offset for these 4 samples compared to the samples from Canada and South Africa may be real or may reflect the larger error in these analyses. Zambales 2005 data were analyzed at the University of Toronto (see text). Kansas data are from 2 continental gas wells and range from −740 to −836‰ (Coveney *et al.*, 1987). Data from the Oman ophiolite seep gases are from Neal and Stanger (1983) (−697‰, −699‰, −699‰, −714‰) and from Fritz *et al.* (1992) (−712‰, −721‰, −733‰).

producing reactions. In the tectonically stable billion-year-old rocks of the Precambrian Shield sites in this study, a magmatic H_2 input is not likely, given noble gas measurements that support a dominantly crustal origin for the dissolved gases (Lippmann *et al.*, 2003; Sherwood Lollar *et al.*, 1993b). However, many of the study sites in the Canadian and Fennoscandian shields are located in Archean greenstone belts where ultramafic rocks in general and serpentinized mineralogies in particular are widespread (Sherwood Lollar *et al.*, 1993a, 1993b). H_2 production coupled to serpentinization is a well-characterized phenomenon (Schulte *et al.*, 2006; Sleep *et al.*, 2004). Hydration of ultramafic rocks forms hydrous silicates (serpentine) and hydroxides via Equation 1 where:

$$Fe_2SiO_4 + 5Mg_2SiO_4 + 9H_2O \rightarrow 3Mg_3Si_2O_5(OH)_4 + Mg(OH)_2 + 2Fe(OH)_2 \quad (1)$$

is coupled with oxidation of Fe^{2+} to form magnetite and H_2 gas via Equation 2:

$$3Fe(OH)_2 \rightarrow Fe_3O_4 + 2H_2O + H_2 \quad (2)$$

At sites such as Kidd Creek in Timmins, Canada, and the Ylivieska, Juuka, and Outokumpu sites in Finland, H_2 production via serpentinization is a likely scenario, given the abundance of ultramafic rocks. The Kidd Creek deposit (2700 Ma) is one of the world's largest massive volcanogenic sulphide deposits and is thought to be an ancient hydrothermal seafloor spreading center, which is reflected in its layered steeply dipping felsic, mafic, and ultramafic units (Bleeker and Parrish, 1996). The Juuka and Outokumpu sites are part of the 1900–2100 Ma Outokumpu ophiolite sequence and are associated with Cu-Ni sulphide ore. The Outokumpu complex consists of serpentinite lenses and enveloping quartz, skarn, and carbonate rocks rimmed

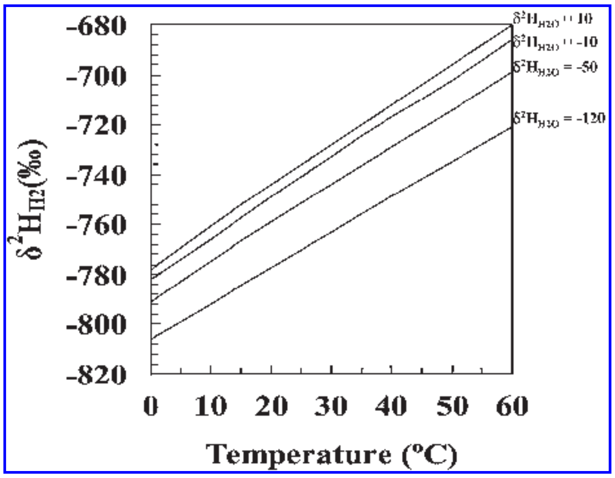

FIG. 3. The y-axis shows the range of expected δ^2H values for H_2 dissolved in groundwater systems with temperatures between 0–60°C and with δ^2H_{H2O} values typical of meteoric groundwaters (+10‰ to −120‰). The calculations indicate that for groundwater residence times that exceed 10^3 to10^5 years (Lin *et al*., 2005b), δ^2H values for H_2 are constrained to the range of −680‰ to −800‰ assuming isotopic re-equilibration between H_2 and H_2O. Values of $\alpha_{H2O(l)\text{-}H2(g)}$ are based on Equation 8 from Horibe and Craig (1995).

by black schists and embedded in mica gneisses and mica schists (Gaal, 1985; Parkkinen and Reino, 1985; Simonen, 1980). The serpentinites are thought to be tectonically emplaced fragments of Precambrian submarine hydrothermal deposits (Gaal, 1985; Papunen and Vorma, 1985). As such, the gases at these sites are a potential ancient analogue for the serpentinite-hosted gases reported at Lost City and other ultramafic-hosted hydrothermal fields on the ocean floor (Charlou *et al*., 2002; Kelley *et al*., 2005).

In contrast, while ultramafic and serpentinized rocks are present in the Witwatersrand Basin, they were not locally extensive at any of the sites investigated by our team. The absence of significant exposures of ultramafic rock, the lack of evidence for serpentinization and the elimination of other possible H_2-producing water-rock reactions such as formation of FeS_2 from FeS (Drobner *et al*., 1990), thermal decomposition of alkanes and carboxylic acids (Seewald, 2001), oxidation of Fe^{2+}-bearing minerals (Stevens and McKinley, 1995), and fracture-induced reduction of water (Kita *et al*., 1982) based on Gibbs free energy calculations lent support to the idea that the production of H_2 in the basin host rocks was a result of an alternative mechanism of water-rock interaction, specifically, radiolytic decomposition of water (Lin *et al*., 2005a, 2005b). Lin *et al*. (2005b) showed that, over the Ma timescale estimated for groundwaters in the Witwatersrand Basin (Lippmann *et al*., 2003), rates of H_2 generation of 0.1–1 n*M*/year are sufficient to produce the observed m*M* concentrations of dissolved H_2 in the groundwaters. Lin *et al*. (2005a) showed that these rates of H_2 generation are consistent with radiolytic decomposition of water sustained by reported U, Th, and K contents of the Witwatersrand Basin host rock.

H_2 in the deep biosphere—balance of sources and sinks

The variety of different H_2-producing water-mineral reactions, as outlined above, that are feasible in the crystalline host rocks of the Precambrian Shield sites of Canada, Finland, and South Africa suggests that significant levels of H_2 in the free gas phase or dissolved in groundwaters in this geologic setting are not at all unexpected. While the specific H_2-producing mechanism will vary from site to site as a function of the local geology, mineralogy, and geochemical history, the long residence times [on the order of millions to tens of millions of years (Lin *et al*., 2006b; Lippmann *et al*., 2003)] for the groundwaters provide a setting in which the products of water-rock reactions can accumulate. The intriguing question in these systems is not so much the source of the H_2, whether from radiolysis, serpentinization, or other H_2-producing reactions, but the mechanism by which it accumulates to such high (m*M*) concentrations. Previous studies have documented the presence of *in situ* microbial communities that couple H_2 utilization to sulfate reduction and, to a lesser extent, to microbial methanogenesis in these ancient formation waters (Lin *et al*., 2006b; Sherwood Lollar *et al*., 2006). Reconciling the presence of H_2 utilizers with m*M* concentrations of dissolved H_2 suggests factors other than substrate availability are limiting microbial activity (Lin *et al*., 2006b). The current paper suggests that fracture-controlled groundwater flow in the crystalline rocks and resulting discontinuous hydraulic connectivity means that mixing between isolated fracture networks is limited and episodic (Frape *et al*., 1984; McNutt *et al*., 1990). A conceptual model is presented that demonstrates how periodic opening of fractures and resulting groundwater mixing controls the distribution and supply of H_2 in this setting.

Fracture-controlled hydrogeology

In crystalline rock, hydrogeologically isolated fractures often contain isotopically and geochemically distinct end members. Previous studies published on the groundwaters of the Canadian Shield demonstrated that the major geochemical and isotopic parameters were controlled by mixing between fresh to moderately saline shallow groundwaters with $\delta^{18}O$ and δ^2H values consistent with paleometeoric waters, and deep ancient groundwaters characterized by very high salinities (up to hundreds of g/L) with $\delta^{18}O$ and δ^2H values that fall to the left of the Global Meteoric Water Line (GMWL) (Frape *et al*., 1984). While the ultimate origin of these fluids may have been saline waters that penetrated the crystalline basement, formation water, or hydrothermal fluids, their geochemical and isotopic signatures have been so profoundly overprinted by the effects of long-term water-rock interaction in these high rock/water ratio fractures that little evidence of their primary composition remains (Fritz and Frape, 1982). The isotopic composition of the groundwaters lies along trends of increasing isotopic enrichment in ^{18}O and ^2H with increasing salinity, which is indicative of significant mixing on a regional scale (Frape *et al*., 1984) controlled by periodic opening of fractures due to tectonic changes. Mixing and precipitation of secondary minerals (primarily calcite and quartz), as well as further tectonic activity, reseal the fractures, which creates pockets of hydrogeologically isolated waters, since the geochemical and isotopic signatures of both waters and fracture minerals can vary substantially even on opposite sides of the same fault system (McNutt *et al*., 1990).

Investigations of groundwater systems in the Witwatersrand Basin of South Africa support a similar model. As shown in Fig. 4, $\delta^{18}O$ and δ^2H values typically describe mixing lines between the most saline, deepest groundwaters with $\delta^{18}O$ and δ^2H values to the left of the GMWL and less saline paleometeoric waters that fall along the GMWL (Ward *et al*., 2004). Temperature histories and age estimates reinforce this end-member mixing scenario. Paleometeoric fluids range in age from approximately 10 Ka to 1–5 Ma, and their temperatures range from 30–40°C . The deeper groundwaters, with salinities and isotopic compositions that reflect their long residence times in the deep subsurface and the prolonged effects of water-rock interaction, have temperatures of 45–60°C and age estimates in the tens of millions of years based on noble gas and ^{36}Cl measurements (Lippmann *et al*., 2003; Onstott *et al*., 2006a; Ward *et al*., 2004).

Figure 4 illustrates that the distribution of H_2 in the Witwatersrand Basin is consistent with this end-member model for the groundwaters. The highest H_2 concentrations are indeed found in the oldest, most saline groundwaters with the most altered $\delta^{18}O$ and δ^2H signatures. Paleometeoric groundwaters falling along the GMWL all have dissolved H_2 concentrations in the low μM to nM range (Fig. 4; Table 1). Sherwood Lollar *et al*. (2006) demonstrated that the oldest, most saline, and H_2-rich groundwaters were also the samples in which the hydrocarbon gas component was dominated by an abiogenic isotopic signature with more ^{13}C-enriched CH_4 and a substantial quantity of C_2+ in addition to CH_4. Groundwater and gas geochemical compositions, and isotopic signatures of both gases and waters, all reflect the effects of extensive water-rock interaction.

Implications of fracture-controlled hydrogeology on biogeochemistry and microbiological activity

Lin *et al*. (2006b) published microbiological and molecular analyses of the *in situ* microbial community in one of the H_2-rich, abiogenic hydrocarbon-dominated saline fracture waters at 2.8 kmbls (kilometers below land surface) in the Mponeng gold mine, for which age estimates of 15–25 Ma had been derived. The samples were both low in biomass (cell density of 10^4 cells/ml) and had exceptionally low biodiversity, dominated by a single phylotype closely related to thermophilic sulfate-reducers. Gibbs free energy calculations were consistent with H_2 utilization by sulfate reducers and, to a lesser extent, methanogens (also identified as a minor phylotype based on 16sRNA clone libraries) sustained by geologically produced SO_4 and H_2 via Equations 3 and 4.

$$4H_2 + H^+ + SO_4^{2-} \rightarrow HS^- + 4H_2O \quad (3)$$

$$4H_2 + H^+ + HCO_3^- \rightarrow CH_4 + 3H_2O \quad (4)$$

Intriguingly, the system was energy-rich and not nutrient-limited, but *in situ* rates of microbial sulfate reduction and methanogenesis were esti-

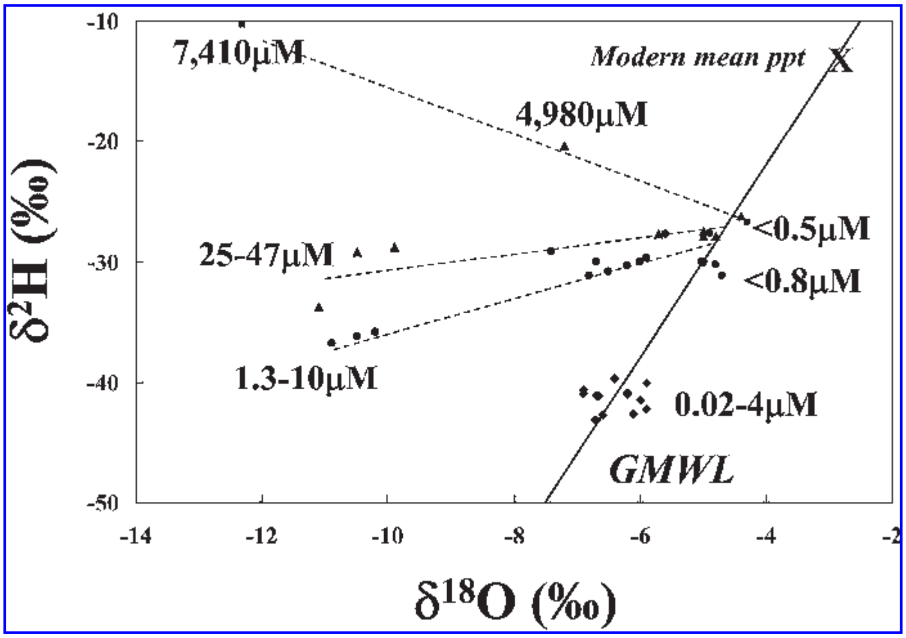

FIG. 4. δ^2H_{H2O} and $\delta^{18}O_{H2O}$ values for groundwaters from the Witwatersrand Basin [data from Ward *et al.* (2004)] with dissolved H_2 concentrations from Table 1. The global meteoric water line (GMWL) is from Craig (1961) and the International Atomic Energy Agency (1981). Possible mixing lines are drawn in to emphasize the trends between samples that lie on the global meteoric waterline and more water-rock-reaction–dominated end-members falling to the left of the GMWL. Symbols represent different mine sites (squares—Driefontein; triangles—Kloof; circles—Evander; diamonds—Beatrix). Locations and detailed geologic descriptions can be found in Ward *et al.* (2004).

mated to be <1 n*M*/year (Lin *et al.*, 2006b) and <0.01 n*M*/ year (Onstott *et al.*, 2006a) respectively, which indicates the likelihood of as-yet-unidentified limiting factors on microbial metabolic activities. Hence, despite the presence of active microbial communities, H_2 concentrations remain high, and CH_4 compositions and isotopic signatures still reflect the abiogenic signature associated with water-rock interaction, which indicates that the contribution of microbial CH_4 must be small relative to the abiogenic component.

In contrast, microbial and molecular studies carried out for the paleometeoric groundwater end members typically exhibit both higher total biomass and greater biodiversity (Lin *et al.*, 2006a; Onstott *et al.*, 2006a; Sherwood Lollar *et al.*, 2006; Ward *et al.*, 2004). Sherwood Lollar *et al.* (2006) noted that the decrease in H_2 observed in these younger, less saline paleometeoric waters was accompanied by a shift in both the isotopic values and composition of the hydrocarbon gases toward signatures consistent with significant production of more ^{13}C-depleted microbial CH_4 via Eq. 4 and an overprinting of the more ^{13}C-enriched and C_2+-rich abiogenic hydrocarbon signature associated with the deeper water-rock interaction dominated end member (Fig. 5). Estimated rates of methanogenesis in these shallower groundwaters (100 n*M*/year) are consistent with this scenario (Onstott *et al.*, 2006a), as subsurface microbial communities access the products of geological water-rock reactions to sustain metabolic activity, which is dominated by H_2 utilization coupled to sulfate reduction and methanogenesis (Lin *et al.*, 2006a; Onstott *et al.*, 2006a). Potentially, the H_2 autotrophs could be auxotrophs (Ladapo and Whitman, 1990) that require an additional growth factor that only becomes available when more saline fracture waters mix with shallower paleometeoric waters.

The Precambrian Shield rocks of Canada, Finland, and South Africa are some of the oldest rocks on Earth and exhibit ages from approximately 2 to more than 3 Ga. Even the oldest groundwaters are several orders of magnitude younger (millions of years) and at some point in the past may have been sterile due to the high-temperature histories of these geological provinces. The presence of active *in situ* microbial communities distinct from surface organisms suggests that, as the system cooled at some point in the geologic past, the deep subsurface was re-

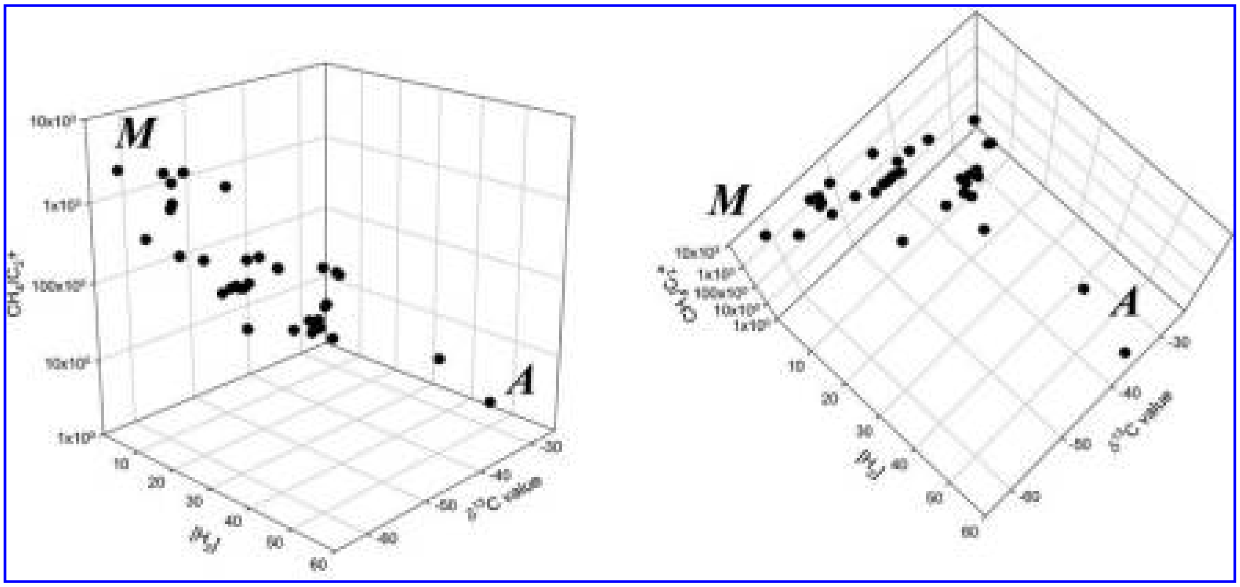

FIG. 5. Dissolved H_2 concentrations (in volume %), CH_4/C_2^+ ratios, and $\delta^{13}C_{CH4}$ values are plotted for sites from the Canadian Shield and Witwatersrand Basin (data from Ward *et al.*, 2004; Sherwood Lollar *et al.*, 2006; and Table 1). The oldest, most saline groundwaters with δ^2H_{H2O} and $\delta^{18}O_{H2O}$ values to the left of the GMWL are also those samples in which the gas compositions and isotopic signatures are dominated by the products of water-rock interaction. High levels of dissolved H_2 are correlated with the most ^{13}C-enriched $\delta^{13}C_{CH4}$ values and the lowest CH_4/C_2^+, ratios consistent with abiogenic hydrocarbon gases derived from water-rock reactions (denoted *A*). As hypothesized in Sherwood Lollar *et al.* (2006), as H_2 levels decrease due to H_2 autotrophy linked to methanogenesis in the paleometeoric end members (see Eq. 4 in text), the increasing contribution of microbial CH_4 results in an overprint of the abiogenic CH_4 signature and a shift to more ^{13}C-depleted $\delta^{13}C_{CH4}$ values and higher CH_4/C_2^+ ratios (denoted *M*).

populated by fracture flow and mixing (Onstott *et al.*, 2006a). The findings of Lin *et al.* (2006b) suggest that such a repopulation may have taken place, for instance, at least 15–25 Ma ago in the Witwatersrand Basin. The substantial geochemical and isotopic heterogeneity of groundwaters and gases, as summarized in the current paper, demonstrates that groundwaters in these crystalline rock settings are not well mixed. As noted in the earliest studies of groundwaters from the Canadian Shield, the system is one best described by episodic hydraulic connectivity and mixing as fractures open, due to tectonic shifts, and close as fractures are resealed due to secondary mineral precipitation and further tectonic activity (McNutt *et al.*, 1990). Figure 6 presents a conceptual model to show how the hydrogeologic constraints of fracture-controlled flow can provide the spatial and temporal discontinuity that allows the products of slow water-rock interaction to build up to high levels over geologic time. As described above, while microbial metabolism via H_2 utilization coupled to sulfate reduction and methanogenesis is thermodynamically feasible in the ancient highly saline fracture waters, the calculated rates for these reactions (<1 nM/year, and <0.01 nM/yr, respectively, for Equations 3 and 4) suggest an as-yet-unidentified limiting factor (Lin *et al.*, 2006b; Onstott *et al.*, 2006a), and the gas geochemistry and isotopic compositions still reflect the dominance of abiogenic processes of water-rock interaction. When the fractures open and mixing with younger paleometeoric waters occurs (Fig. 4), the pattern shifts to one of H_2 depletion and hydrocarbon gas chemistry and isotopic signatures consistent with H_2 utilization and methanogenesis (Sherwood Lollar *et al.*, 2006), *i.e.*, geochemical and isotopic observations that reflect the overprinting of abiogenic processes by microbial activity. These geochemical and isotopic observations are supported by the shift in the microbial community in these boreholes toward increasing biodiversity, increased biomass, and a higher proportion of methanogens (Ward *et al.*, 2004) compared to the highly saline fracture end member described by Lin *et al.* (2006b). These processes may have occurred on a spectrum of timescales—over Ma timescales due to natural processes of strain and deformation, but also and more recently due to changes induced by mine drilling and exploration.

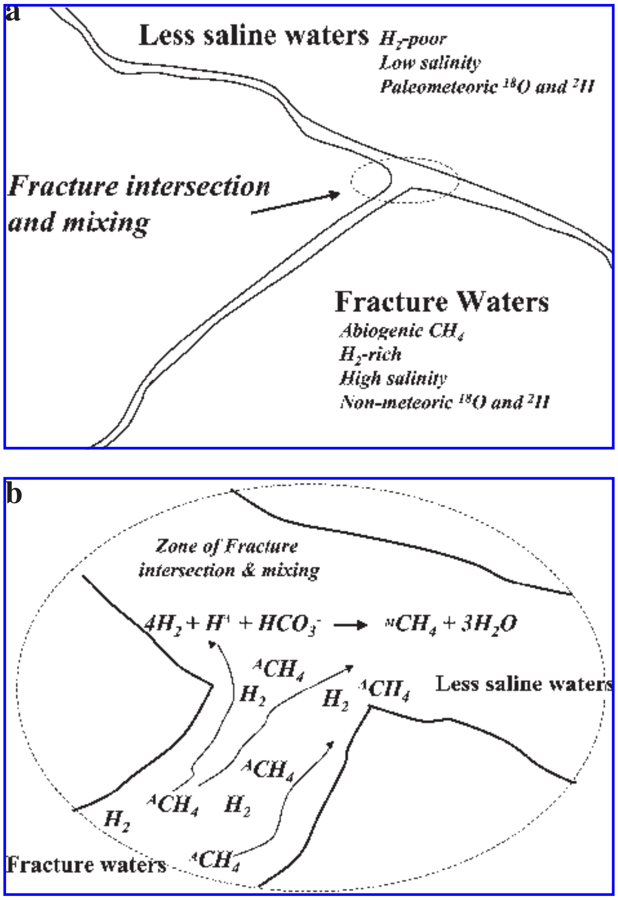

FIG. 6. (a) Conceptual model for hydrogeologically isolated fracture-controlled groundwater flow and mixing in crystalline rock. In sealed fractures, products of abiogenic water-rock reactions accumulate over geologically long timescales (H_2, $^{A}CH_4$). Episodic fracture opening mixes highly saline groundwaters with younger paleometeoric waters to generate the trends shown in Fig. 4 and Figs. 5a and 5b. (**b**) Detail of fracture intersection and mixing zone. This schematic does not attempt to depict all geochemical reactions taking place and does not include all parameters. It is shown to illustrate how the observed H_2 depletion and shift to microbial methane signatures might arise due to the hypothesized mechanism of fracture intersection and mixing. H_2 depletion is accompanied by production of a second microbial CH_4 component ($^{M}CH_4$) that mixes with and overprints the abiogenic CH_4 signature ($^{A}CH_4$).

We suggest that the end-member distribution of major geochemical parameters, the isotopic signatures for groundwaters, the compositional and isotopic signatures of dissolved gases, and the microbiological composition and biomass in the deep fracture waters of the Precambrian Shield are a function of long-term accumulation of the products of water-rock interaction and energy substrates, combined with limited microbial activity in the deepest groundwaters. Based on these observations, our conceptual model is that the hydrogeologically isolated fracture-controlled groundwater system periodically releases H_2 that drives deep subsurface microbial activity. The timescales of these episodic releases and of the resulting microbial activity remain to be determined, but geodynamic models by Sleep and Zoback (this volume) suggest the timescales of rock faulting and deformation (10^5 to10^6 year) are consistent with the noble-gas–derived residence times for the groundwaters described in this paper. The geologically stable ancient Precambrian cratons of Earth are the closest analogues available to single-plate planets such as Mars. While hydrothermal vents have been suggested as target sites for exploration for life on Mars, the degree of recent volcanism and hydrothermal activity is still debated (Krasnopolsky, 2005; Lyons *et al.*, 2005), and no extinct or extant vents have been positively identified to date. Given that strain rates in the martian crust have been suggested to be of the same order of magnitude as those on the terrestrial cratons (Sleep and Zoback, 2007), this paper suggests that H_2 derived from alteration of the martian basaltic crust might be sequestered in fractures and episodically released in a system analogous to the terrestrial Precambrian cratons. This implies that the habitability of the martian crust might not be restricted to sites of localized hydrothermal activity. While the presence of the martian cryosphere and potential clathrates will affect the porosity and permeability of the martian crust, as well as the net flux of gases (Clifford and Parker, 2001; Onstott *et al.*, 2006b), the underlying principles of fracture-controlled energy sequestration and episodic release cannot be ignored. Ongoing investigation by this team of researchers into fracture-controlled geologic energy for life in Precambrian Shield sites in the Arctic where permafrost and clathrate deposits are present will provide additional constraints on habitability of Earth's deep "cold" biosphere and important implications for the search for life on Mars.

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ABBREVIATIONS

GC, gas chromatograph; GMWL, Global Meteoric Water Line; LCHF, Lost City Hydrothermal Field.

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