Evidence of life on Earth is manifestly preserved in the rock record. However, the microfossil record only extends to ~3.5 billion years (Ga), the chemofossil record arguably to ~3.8 Ga, and the rock record to 4.0 Ga. Detrital zircons from Jack Hills, Western Australia range in age up to nearly 4.4 Ga. From a population of over 10,000 Jack Hills zircons, we identified one >3.8-Ga zircon that contains primary graphite inclusions. Here, we report carbon isotopic measurements on these inclusions in a concordant, 4.10 ± 0.01-Ga zircon. We interpret these inclusions as primary due to their enclos-ure in a crack-free host as shown by transmission X-ray microscopy and their crystal habit. Their δ13C graphite of −24 ± 5% is consistent with a biogenic origin and may be evidence that a terrestrial biosphere had emerged by 4.1 Ga, or ~300 My earlier than has been previously proposed.

Hadean | carbon isotopes | early Earth | zircon | origin of life

Life on Earth is an ancient phenomenon, with the earliest identified microfossils at nearly 3.5 billion years before present (Ga) (1) and the earliest potential chemofossils at 3.83 Ga (2, 3). Investigation of older materials is limited by the increasingly sparse and metamorphosed rock record, with the oldest rock age at 4.0 Ga (ref. 4; cf. ref. 5). Given the temporal limits of the rock record, it has been difficult to assess terrestrial habitability or document a biosphere before ~3.8 Ga. Despite the lack of a rock record before 4.0 Ga, detrital zircons as old as 4.38 Ga have been documented (6). About 5% of the zircons from a ~3-Ga metagraywacke at Jack Hills, Western Australia yield ages greater than 3.8 Ga. Their geochemistry and mineral inclusions have been interpreted to indicate their derivation largely from hydrous, sediment-derived granitic magmas (7–12). The relatively clement conditions implied suggest a potentially habitable planet and leave open the possibility of a Hadean (>4-Ga) biosphere.

The stable isotopic ratio δ13C/12C (herein defined relative to the Pee Dee Belemnite standard, i.e., δ13C_PDB) provides a potential biosignature due to the isotopic fractionation that occurs during carbon fixation. As a consequence, biogenically derived kerogens yield an average of −25 ± 10‰ across the sedimentary rock record from 3.4 Ga to the present (13), whereas carbonates and mantle values are consistently offset with averages of 0‰ (13) and ~5‰ (14), respectively. Thus, the discovery of isotopically light graphite in Eoarchean metasediments from southern West Greenland (2, 15) was proposed as evidence of a 3.7-Ga 3.8-Ga biosphere.

The >3.8-Ga Jack Hills zircons contain abundant mineral inclusions, mostly of a granitic character (11). Abundant and intimately associated diamond and graphite (4% of each in the zircons investigated) were reported in previous studies (16, 17) but subsequently shown to be diamond polishing debris and epoxy that had lodged in cracks during sample preparation (18). This left the true occurrence and abundance of carbonaceous materials in the Jack Hills zircons uncertain. We imaged a large number of >3.8-Ga Jack Hills zircons in search of carbonaceous inclusions and made carbon isotopic measurements of primary graphite found in one grain. To our knowledge, we report here the first unambiguous carbon isotopic measurements of terrestrial Hadean material.

Results

From an initial population of over 10,000 Jack Hills zircons (6), we examined 656 grains with ages over 3.8 Ga for the presence of graphitic inclusions. The zircons were mounted in epoxy and polished to expose their interiors. The search protocol included an initial screening for opaque inclusions using transmitted light microscopy. Seventy-nine candidates thus identified were then targeted for Raman spectroscopy from which we documented two zircons containing partially disordered graphite (Fig. 1, In-

Figure

Significance

Evidence for carbon cycling or biologic activity can be derived from carbon isotopes, because a high δ12C/13C ratio is characteristic of biogenic carbon due to the large isotopic fractionation associated with enzymatic carbon fixation. The earliest materials measured for carbon isotopes at 3.8 Ga are isotopically light, and thus potentially biogenic. Because Earth’s known rock record extends only to ~4 Ga, earlier periods of history are accessible only through mineral grains deposited in later sediments. We report 12C/13C of graphite preserved in 4.1-Ga zircon. Its complete encasement in crack-free, undis-
turbed zircon demonstrates that it is not contamination from more recent geologic processes. Its 12C-rich isotopic signature may be evidence for the origin of life on Earth by 4.1 Ga.

Author contributions: E.A.B., P.B., and T.M.H. designed research; E.A.B., P.B., and T.M.H. performed research; W.L.M. contributed new reagents/analytic tools; E.A.B., P.B., and T.M.H. analyzed data; and E.A.B., P.B., and T.M.H. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

1To whom correspondence may be addressed. Email: ebell21@ucla.edu or tmark.harrison@gmail.com.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1517557112/-/DCSupplemental.

Elizabeth A. Bella1, Patrick Boehnke2,3, T. Mark Harrison1,4, and Wendy L. Mao5

1Department of Earth, Planetary, and Space Sciences, University of California, Los Angeles, CA 90095; and 2School of Earth, Energy, and Environmental Sciences, Stanford University, Stanford, CA 94305

Contributed by T. Mark Harrison, September 4, 2015 (sent for review July 31, 2015)

PNAS Early Edition | 1 of 4

www.pnas.org/cgi/doi/10.1073/pnas.1517557112
The zircon sliver was remounted in indium, coated with gold, and analyzed for $\delta^{13}C$ on a Cameca ims 1270 secondary ion mass spectrometer (SIMS) using a 5-nA Cs$^+$ primary beam (21) with static multicollection following a 2-min cleaning via rastering of the primary beam. Note that the two inclusions had not previously been exposed to epoxy or any other source of laboratory carbon contamination and were revealed only by drilling with the primary Cs$^+$ ion beam. During analysis of the smaller inclusion, we found that count rates increased to a maximum value as the inclusion was breached followed by a tailing to background (Supporting Information). This precludes the possibility that our data were affected by surface contamination.

SIMS measurement of C isotopes in carbonaceous materials is known to be little affected by instrumental mass fractionation. For example, a previous study found less than 2‰ variation in an intercomparison of hydrocarbon standards that varied in H/C by a factor of 7 (21). Thus, we used epoxy, with $\delta^{13}C$ of $\sim-26.8‰$ (21), as a standard material for instrumental mass fractionation corrections and Escherichia coli with $\delta^{13}C$ of $\sim-24‰$ (21) as a secondary standard. The two inclusions yielded similar results in both carbon abundance and $\delta^{13}C$. Maximum signals of $\sim10^5$ counts per second (cps) of $^{12}C_2^+$ for the inclusions was at least an order of magnitude higher than for the C background in the indium mounting material ($\sim10^4$ cps) and much higher than a clean area in the zircon ($\sim10^3$ cps). The $\delta^{13}C$ values of the two inclusions are indistinguishable within error, and thus we combined the results to obtain an average of $\sim-24 \pm 5‰$. This is notably light with respect to terrestrial inorganic carbon and most meteoritic carbon, but consistent with terrestrial biogenic carbon (Fig. 2). Following carbon isotopic analysis, we measured rare earth element (REE) and Ti abundances using the ims 1270 with a 15-nA O$^-$ primary beam. Two spots yielding measurable Ti correspond to an average apparent crystallization temperature of $\sim660$ °C, similar to the average Hadean Jack Hills value (9), with REE consistent with crystallization under reduced conditions (using the method of ref. 22) and a continental trace element signature (following the discriminant diagrams of ref. 23).

Fig. 1. Transmission X-ray image of RSES 61-18.8 with graphite indicated. (Inset) Raman spectra for the top inclusion and for an epoxy "inclusion" from another investigated zircon. The broadened "D-band" at $\sim1,400$ cm$^{-1}$ indicates disordered graphite (39); C–H stretch bands at $\sim2,800$–$3,100$ cm$^{-1}$ (39) are observed in epoxy but not graphite.

Fig. 2. $\delta^{13}C$ for Eoarchean–Hadean carbon samples measured via SIMS vs. host mineral age compared with inorganic and organic carbon (organic carbon values from ref. 13; inorganic from ref. 14).
Discussion

Given the isolation of these inclusions provided by their entrapment in a crack-free and isotopically undisturbed zircon crystal, as well as the vanishingly slow self-diffusion of carbon in graphite under crustal conditions (24), we interpret them to be petrologically primary and isotopically closed systems. Because these inclusions are at least as old as their 4.10-Ga host, the observation of isotopically light carbon raises the possibility that biologic processes were operating during the Hadean. To be sure, not all light carbon isotopic signals are biogenic.

Abiotic processes that could produce light δ13C during the Hadean include Fischer–Tropsch mechanisms (25) and carbon isotopic fractionation by diffusion (26), incorporation of meteoritic carbon (ranging from +68‰ to −60‰ δ13C) materials, mid-ocean ridge basalt degassing (27), and high-temperature disproportionation of siderite (28). However, in the absence of experimental evidence for particulate carbon formation via a Fischer–Tropsch process (ref. 25; cf. ref. 27) or diffusive or mineral disproportionation mechanisms that could selectively lead to light carbon condensates that were ultimately incorporated in a low-temperature (i.e., ∼650 °C) granitoid zircon, a biogenic origin seems at least as plausible. The quantity of extraterrrestrial carbon necessary to dominate the carbon budget of a felsic magma protolith would be significant and profoundly influence the chemistry of that magma away from its granitoid character unless separated from the host meteorite. A possible mechanism to effect this on ancient Earth might be through sedimentary processing and concentration of meteoritic carbon in pelagic sediments. Even if meteoritic carbon could be concentrated in this fashion, our observed isotopic signal is uncharacteristic of the most likely candidates, carbonaceous chondrites, which contain on average 3.5 wt% C (29). Although δ13C of various components ranges between +68‰ and −34‰ (30), only 10% of bulk carbon measurements have δ13C less than −29‰ (31), and thus this class of meteorites would be a fortuitous, although possible, source for both inclusions. Our view that isotopically light C in ancient terrestrial materials may be of biogenic origin is consistent with interpretations of light δ13C in mantle diamonds (e.g., ref. 32). Although diamonds as low as approximately −10‰ are able to be accounted for by isotopic fractionation of abiogenic precursors, more negative values down to −23‰ do not appear to be explicable by such processes and strongly suggest an origin from subducted organic matter (33). Although we cannot rule out an abiogenic source, the eventual acquisition of a large database of Hadean carbon isotope measurements will permit the plausibility of hypothetical abiotic origins to be more closely scrutinized.

By contrast, biogenic organic matter is incorporated into the vast majority of preserved clastic sediments, can reach 10% by weight in Phanerozoic sediments (34), and has averaged δ13C approximately −25‰ over the past 3.5 Ga (13). Given this historical trend and the several lines of evidence indicative of sediment involvement in the Jack Hills zircon magmas (8, 10–12), the simplest interpretation of our data are that the carbonaceous inclusions represent graphitized organic carbon present during melting of a pelitic protolith at 4.10 Ga. This interpretation is entirely consistent with the observed low crystallization temperature, continental affinity, and reduced environment (35) of zircon formation.

The isolated, primary nature of the graphite in sample RSES 61-18.8, along with the lack of diamond features in its Raman spectrum, differs from the abundant graphite previously reported (16, 17) in Jack Hills zircons, which was found often intersecting cracks in the host zircon, and all of which were reported to contain diamond (17). As noted, the diamonds were later found to be contamination (18). The rare occurrence of primary graphite in this study (in <0.2% of >3.8-Ga zircons) also contrasts with these earlier results (graphite in 4% of zircons of all ages), further suggesting that much or all of the earlier-purported graphite identified was contamination.

There are considerable limitations of basing any inference regarding early Earth on a single zircon containing primary carbonaceous inclusions. Instead, we see this contribution as demonstrating the feasibility of perhaps the only approach that could lead to establishing a Hadean carbon isotope record. In this regard, we emphasize that because >3.8-Ga grains make up only ~5% of the Jack Hills zircon population (6), RSES 61-18.8 represents an abundance of carbon-bearing Jack Hills zircons of only about 1-in-10,000.

Conclusions

This study extends the terrestrial carbon isotope record ~300 My beyond the previously oldest-measured samples from southwest Greenland. Our interpretation that the light C isotope signature in primary graphitic inclusions could reflect biologic processes is consistent with an estimate from molecular divergence in prokaryote phylogenetic relationships that a terrestrial biosphere had emerged by 4.1 Ga (36). Confirming such a connection would represent a potentially transformational scientific advance. However, given the low occurrence of carbon-bearing Hadean zircons, establishing a Hadean carbon cycle and its possible bearing on the origin of life will require enormous and sustained efforts.

Methods

Zircons were mounted in epoxy and polished to expose their interiors, typically involving loss of approximately one-third of the zircon during polishing. Polishing was accomplished with 1,200-grit silicon carbide paper and 1-μm diamond paste. The search protocol included an initial screening for opaque inclusions using transmitted light microscopy and a 40× objective lens.

Raman Spectroscopy. Confocal Raman spectroscopy was carried out on zircons mounted in epoxy in several laboratories, using a green laser and 20× or 40× objective lenses. Opaque inclusions noted by transmitted light microscopy were analyzed for carbonaceous material. This screening procedure includes some inherent bias toward zircons clear enough for effective transmitted light microscopy, although this appears to include most of the Hadean Jack Hills population imaged in this study. We also note that our screening protocol does not completely preclude some of the other opaque inclusions from containing some carbonaceous material—the Raman signal could be masked by high noise.

FIB Milling. Sample RSES 61-18.8 was coated in gold and a ∼60 × 30 × 20 triangular prismatic region containing the identified carbonaceous inclusion was milled from the larger zircon sample using a Nova 600 SEM/FIB system at the University of California, Los Angeles (UCLA) Nanoelectronics Research Facility. The section of zircon was attached to a tungsten needle using a platinum weld.

X-Ray Microscopy. The FIBbed section of RSES 61-18.8 was imaged by transmission X-ray microscopy at beam line 6-2c of the SSRL (19), at a spatial resolution of 40 nm. An energy of 7,160 eV was used for imaging. One X-ray image is seen in Fig. 1, while movies constructed from the X-ray slices are presented in Supporting Information.

Carbon Isotopic Measurements. We used the method of ref. 3, modified for static multicollection. We also included a 2-min precleaning of analysis surfaces with a 20 × 20-μm raster of the primary beam. We used a ≳ primary beam of 5 nA for measurements on E. coli and zircon samples and of 2 pA for measurements on the epoxy. We used epoxy as our primary standard (−26.8‰ PDB; ref. 21) for all instrumental mass fractionation corrections and E. coli as a secondary standard (−24.1; our analyses averaged −19 ± 4‰).

Error bars on our graphite inclusions incorporate the internal error bars for the analyses, the reproducibility of the epoxy, and quadratic addition of a 4‰ uncertainty due to potential matrix effects between epoxy and graphite (21). The latter is included even though negligible matrix effects were noticed in an earlier study, where less than 2‰ variation was observed in an internal comparison of hydrocarbon standards that varied in δ13C by a factor of 7 (21). Considering these error bars, normalization to either the primary epoxy or secondary E. coli standard produces statistically indistinguishable δ13C values for our inclusions.
Trace Element Measurement. These analyses were carried out using a Cameca IMS1270 ion microprobe at UCLA, with primary O beam intensity of ~15 nA and a spot size of 60 μm. Secondary ions were detected at high energy offset (~100 eV) to suppress molecular interferences. Further peak stripping for background and isobaric interference corrections was accomplished off-line. Standardization was to the National Institute of Standards and Technology 610 standard glass, with the 91500 zircon (37) used as a secondary standard. Additional correction of (Ti) used a value for 91500 of 5.2 ± 0.3 ppm (38).

ACKNOWLEDGMENTS. Raman spectroscopy by Anatoliy Kudryavtsev, Anke Watenpulhu, Olivier Beyssac, and Daniel Wilkinson, using confocal Raman systems in the laboratories of William Schopf, Andrew Steele, Olivier Beyssac, and Bruce Dunn, made possible our search for carbonaceous inclusions. FIB milling by Noah Bodzin, X-ray imaging by Wendy Mao and Christoper Sneed, and X-ray image reconstruction and for Movie S2. We thank the three reviewers, whose comments greatly improved our manuscript. This project was funded by a Simons Collaboration on the Origin of Life Postdoctoral Fellowship (to E.A.B.) and NSF-EAR Grant 0948724 (to T.M.H.). The UCLA ion microprobe facility is partially supported by a grant from the Instrumentation and Facilities Program of EAR, NSF. Data reported in this manuscript are presented in full in Supporting Information. Portions of this research were carried out at the SSRF, a Directorate of SLAC National Accelerator Laboratory and an Office of Science User Facility operated for the US Department of Energy, Office of Science by Stanford University.