Carbon isotopic composition of individual Precambrian microfossils

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ABSTRACT

Ion microprobe measurements of carbon isotope ratios were made in 30 specimens representing six fossil genera of microorganisms petrified in stromatolitic chert from the ~850 Ma Bitter Springs Formation, Australia, and the ~2100 Ma Gunflint Formation, Canada. The $\delta^{13}C_{PDB}$ values from individual microfossils of the Bitter Springs Formation ranged from –21.3 ± 1.7‰ to –31.9 ± 1.2‰, and the $\delta^{13}C_{PDB}$ values from microfossils of the Gunflint Formation ranged from –32.4 ± 0.7‰ to –45.4 ± 1.2‰. With the exception of two highly ^{13}C-depleted Gunflint microfossils, the results generally yield values consistent with carbon fixation via either the Calvin cycle or the acetyl-CoA pathway. However, the isotopic results are not consistent with the degree of fractionation expected from either the 3-hydroxypropionate cycle or the reductive tricarboxylic acid cycle, suggesting that the microfossils studied did not use either of these pathways for carbon fixation. The morphologies of the microfossils suggest an affinity to the cyanobacteria, and our carbon isotopic data are consistent with this assignment.

Keywords: Precambrian microfossils, Calvin cycle, carbon isotopes, kerogen.

INTRODUCTION

Unlike the familiar Phanerozoic history of megascopic life, evolution during earlier and much longer Precambrian time was characterized by development of metabolic capabilities in microscopic prokaryotes (Schopf, 1994). Because modern prokaryotes are highly diverse physiologically and those having similar appearances can differ markedly in metabolism, the sorting out of such capabilities among Precambrian fossil microorganisms requires more than traditional morphology-based paleontology. To date, this need has been addressed by isotopic analyses of whole rock acid-resistant carbonaceous residues (kerogens), studies that have traced isotopic signatures of biological carbon fixation to ~3500 Ma (Hayes et al., 1992; Schidlowski et al., 1983) and possibly earlier (Schidlowski, 1988), and evidently of both methanogenic and methanotrophic microbes to ~2700 Ma (Hayes, 1994).

Although useful, isotopic analyses of bulk samples cannot provide information specific to the apparent physiology of, or the phylogenetic relations among, individual microscopic fossils. However, because the metabolic pathways that typify different groups of autotrophic prokaryotes can fractionate carbon isotopes by differing amounts—for example, the acetyl-CoA pathway can yield substantially greater fractionation than the Calvin cycle (House, 1999; Preuβ et al., 1989), and the Calvin cycle more than the reductive tricarboxylic acid (TCA) cycle (House, 1999; Preuβ et al., 1989)—isotopic evidence of metabolism and phylogenetic affinities might be preserved in the cell walls and other kerogenous constituents of fossil microorganisms. If so, accurate, 1‰–2 ‰ precision measurements of the carbon isotopic composition of individual Precambrian microfossils could provide significant new means to more clearly define the composition and evolutionary history of the early biota.

The ion microprobe can be used for in situ analysis of the isotopic composition of very small amounts of carbonaceous material (Ireland, 1995; McKeegan et al., 1985). This instrument was used by Mojzsis et al. (1996) for carbon isotopic analysis of individual ~5 µm carbonaceous inclusions hosted within apatite crystals from ~3800 Ma banded iron formation (BIF) sediments. That pioneering study identified isotopically light carbon and inferred fractionation of sufficient magnitude to suggest the presence of biological activity at the time of sedimentation. However, the analytical uncertainties of individual analyses were typically too large (~5‰) to confidently reveal diversity in the isotopic record and, moreover, the BIF studied was severely metamorphosed, eliminating the potential for structural preservation of microfossils. Here we report results of analyses of microorganisms cellularly petrified in two Precambrian microbial communities, those of the Bitter Springs (~850 Ma) and Gunflint (~2100 Ma) microbiotas. Techniques used are such that isotopic measurements have been made with sufficient accuracy and precision to reveal potential isotopic variability in the fossil record on the level of a single microscopic organism.

METHODS

The University of California, Los Angeles, CAMECA ims 1270 ion microprobe was used to analyze the carbon isotopic composition of individual microfossils exposed at the upper surfaces of polished petrographic thin sections (~100 µm thick) of subgreenschist facies stromatolitic carbonaceous cherts from the ~850 Ma Bitter Springs Formation of central Australia (Schopf, 1968) and the ~2100 Ma Gunflint Formation of southern Canada (Barghoorn and Tyler, 1965). Optical microscopy was used to select microfossils of diverse morphology and free of detectable impurities such as carbonate or hematite. Secondary C^- ions were sputtered by a ~10- to 15-µm-diameter Cs^+ beam from the microfossils (Figs. 1 and 2) and analyzed at a mass resolving power between 3000 and 4000 in order to eliminate molecular ion (hydride) interferences. Instrumental mass fractionation was calibrated by interposing analyses of individual microfossils with analyses of a carbonaceous chert specimen from the Precambrian Swaziland Supergroup (Precambrian Paleobiology Research Group [PPRG] sample 215-1), a "standard" that is mineralogically similar to the fossiliferous samples and contains kerogen of

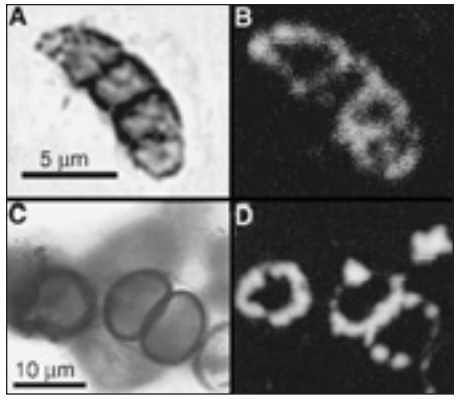

Figure 1. Optical photomicrographs (A and C) and $^{12}C^-$ scanning ion images (B and D) of *Cephalophytarion* (A and B), a filamentous (oscillatoriacean) cyanobacterium, and *Myxococcoides* (C and D), cyanobacterial (Chroococcacean) unicells clustered in a sheath, exposed at upper surface of polished petrographic thin section of stromatolitic Bitter Springs chert. Ion images show that carbon analyzed is derived predominately (>90%) from microfossils rather than from particulate kerogen diffused throughout mineral matrix.

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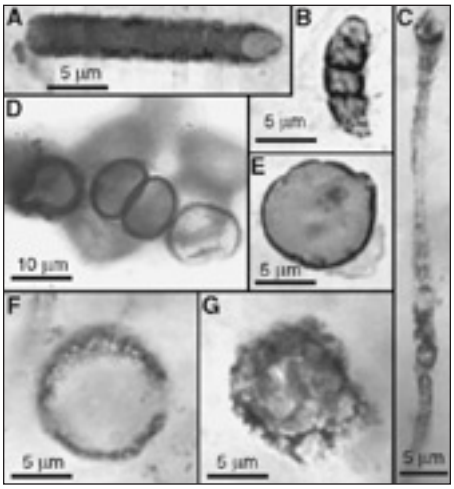

Figure 2. Transmitted-light optical photomicrographs of microfossils for which $\delta^{13}C_{PDB}$ values are listed in Table 3, (a, e, n, o, r, z, and ad). From the Bitter Springs Formation: (A) *Eomycetopsis*; (B) *Cephalophytarion*; (D) *Myxococcoides*; and (E) *Glenobotrydion*. From the Gunflint Formation: (C) *Gunflintia*; (E) *Huroniospora*; and (F) *?Huroniospora*.

TABLE 1. INSTRUMENTAL MASS FRACTIONATION FOR NON-CHERT CARBON-RICH MATERIALS

Sample name	Material	H/C (kerogen)	$\delta^{13}C_{PDB}$ (‰)	$\delta^{13}C_{raw}$ (‰) (uncorrected)	Instrumental Mass Fractionation (‰)	Number of Analyses
SESSION ON MAY 15th, 1996						
PPRG #182-1	Kerogen	0.12	-31.6 ± 0.2	-69.0	-38.6±0.9	3
PPRG #447-1	Kerogen	0.26	-28.4 ± 0.2	-65.8	-38.5±0.3	4
PPRG #1357	Kerogen	0.79	-27.2 ± 0.2	-63.8	-37.2±0.4	3
SESSION ON FEBURARY 21st, 1997						
Epon 828 & TETA	Epoxy	ND	-26.8 ± 0.5	-64.4	-38.4±0.2	12
Graphite Rod	Graphite	0.00	-25.6 ± 0.2	-56.2	-31.0±0.2	7
Greta algal coal	Coal	ND	-14.6 ± 0.2	-54.9	-41.3±0.4	7

Note: $\delta^{13}C_{PDB}$ compositions are either from Hayes et al. (1983) or were measured for this study by conventional means. $\delta^{13}C_{raw}$ (‰) is the uncorrected carbon isotopic composition measured by the ion microprobe. The error listed has been adjusted to reflect the spot-to-spot reproducibility of the analyses of each material, but not to include the uncertainty on the conventional analyses. The algal coal is from the Middle Permian of New South Wales, Australia.

known isotopic composition (Hayes et al., 1983). An ~2-mm-diameter chip of the standard was embedded into each 2.54-cm-diameter thin section in order to facilitate calibration under identical instrumental operating conditions.

Microfossils were analyzed for carbon isotopic composition, using both the monocollection mode of the ion microprobe with magnetic field peak switching, using a 0.5–1 nA primary beam and a single electron multipler detector, and the multicollection mode of the ion microprobe with a static magnetic field, using ~3 nA primary beam and both an electron multipler and a Faraday cup detector. In both types of analysis, results were calibrated by repeated measurements of PPRG sample 215-1; the measurements were used to deduce the appropriate correction for the instrumental mass fractionation and analytical uncertainty for single microfossil measurements. The reported results are the mean $\delta^{13}C_{PDB}$ of repeated analyses of individual fossils ±1 standard error of the mean (1 σ_m). During monocollection analyses, the repeated fossil measurements were conducted throughout the ion microprobe session and were compared to the totality of standard measurements conducted in the same session; no significant isotope effect associated with these repeated analyses was detected. In contrast, during analyses made in the multicollection mode, a small but discernible change in isotopic fractionation was observed when single spots were measured repeatedly. Therefore, each fossil was analyzed only twice, by two short-duration (each a couple of minutes) back-to-back measurements bracketed by repeated analyses of the standard. In comparison with analyses made in the monocollection mode, multicollection analyses were shorter, counted a much larger number of ions, and, because fluctuations in the secondary C^- beam intensity lead to reduced precision when only one collector is used, generally yielded better precision.

RESULTS AND DISCUSSION

Tests of the Analysis Method

Several tests were performed to assess the reproducibility and accuracy of individual analyses. First, during a single ion microprobe session, the instrumental mass fractionation was determined for three kerogen samples of varying H/C composition isolated from Precambrian cherts (PPRG samples 447-1, 182-1, and 1357). The purpose of these experiments was to determine the magnitude of possible differences in the instrumental mass fractionation (matrix effect) for kerogens of differing H/C content. The results, listed in Table 1, indicate that any such matrix effect is <2‰ for kerogens with H/C contents ranging from 0.79 to 0.12.

The instrumental mass fractionation was also measured (during a single ion microprobe session) for several nonchert carbon-rich materials—epoxy, graphite, and an algal coal (Table 1). An approximately 10‰ difference was observed between the instrumental mass fractionation of graphite and coal, a difference that demonstrates the need for use of a standard as chemically similar as possible to the sample analyzed. In the work presented here, the measurements of microfossils (petrified in chert) are compared to organic carbon preserved in another Precambrian chert; the standard and the sample are chemically very similar.

In addition, the accuracy of the microfossil analysis method was tested by comparison of repeated ion microprobe measurements of the carbon isotopic compositions of carbonaceous materials in other rock specimens having $\delta^{13}C$ values determined by conventional whole-rock analyses. The results listed in Table 2 show that the method is effective for determining the carbon isotopic composition of the organic carbon in both cherts and shales, but that an error of ~4‰ can result in the measured $\delta^{13}C$ of graphite when instrumental mass fractionation is calibrated by analysis of the PPRG 215-1 standard. In three of the experiments on cherts or shales, the results are within 1.5‰ of the $\delta^{13}C_{PDB}$ composition determined by conventional mass spectrometry and the precision of multiple analyses is ≤1‰. However, for analyses on PPRG 182-1, the error and the mean squared weighted deviation (MSWD), or reduced χ^2 (see Bevington and Robinson, 1992, p. 72), were larger owing to the sample being heterogeneous in carbon isotopic composition. The lack of agreement for the graphite analyzed is probably due to a matrix effect arising from chemical differences between the graphite rod and kerogenous organic matter preserved in a SiO_2-rich matrix.

These tests show that the chemical differences between different kerogens do not lead to strong matrix effects (<2‰) and thus validate the general analysis method used here to study the carbon isotopic composition of individual ancient microfossils.

Microfossil Results

Fifteen microfossils from the Bitter Springs Formation were analyzed (Table 3, a through o) representing four genera (Figs. 1 and 2). An additional 15 fossils, representing at least two genera (Fig. 2), were analyzed from the Gunflint Formation (Table 3, p through ad). The $\delta^{13}C_{PDB}$ values of all but three of these 30 microfossils fall within the ranges (~10‰) reported for the whole-rock total organic carbon (TOC) of cherts from their respective formations (Fig. 3). Although this reported variability of TOC values could be due in part to contamination by indigenous carbonate, the ion microprobe measurements confirm that the kerogenous carbon of these sediments is isotopically heterogeneous. In the ion microprobe measurements, the vast majority (>90%, typically 95%) of the analyzed carbon comes directly from the preserved microfossil and not from a diffuse background of amorphous kerogen within the chert. Additionally, the carbon isotope signal is uncontaminated by small amounts of fine-grained

TABLE 2. CARBON ISOTOPIC COMPOSITIONS OF CARBON RICH MATERIALS MEASURED BY ION MICROPROBE USING PPRG 215-1 AS A STANDARD

Sample name	Material	H/C (kerogen)	$\delta^{13}C_{PDB}$ (‰) (expected)	$\delta^{13}C_{PDB}$ (‰) (observed)	Calculated Error ($1\sigma_m$)	Number of Analyses	MSWD*
PPRG #022-2†	Shale	~0.1	-31.5 ± 0.2	-31.4	0.6	8	0.2
PPRG #181-1†	Shale	0.02	-15.2 ± 0.4	-13.7	0.5	22	1.3
PPRG #182-1	Chert	0.12	-31.5 ± 0.2	-29.3	2.4	4	2.3
PPRG #215-1	Chert	0.16	-31.5 ± 0.2	-31.1	1.0	4	0.8
Graphite Rod†	Graphite	0.00	-25.6 ± 0.2	-30.1	0.5	16	1.5

Note: PPRG sample 215-1 is from the upper greenschist facies of the ~3,350 Ma Fig Tree Group of South Africa and is reported to have an H/C ratio of 0.16 and $\delta^{13}C_{PDB}$ values of -31.5 ± 0.2‰ for total organic carbon and -31.9 ± 0.1‰ for acid-resistant kerogen (Hayes et al., 1983). "Expected" $\delta^{13}C_{PDB}$ compositions in the table are either from Hayes et al. (1983) or were measured for this study by conventional means. The analytical uncertainty of each individual microfossil analysis was based on the internal precision of the analysis adjusted to reflect the reproducibility of analyses of the standard. The reported "observed" $\delta^{13}C$ compositions are the weighted mean of multiple individual analyses ±1 standard error of the mean ($1\sigma_m$).

*Mean Square Weighted Deviation (MSWD) = $\sum_{i=1}^{n}([X_{wm} - X_i]^2 / \sigma_i^2) / (n-1)$

where X_{wm} = weighted mean of the X_i, σ_i = 1 standard error in measurement of X_i, and n = # of measurements.

†Specimens analyzed using multicollection mode.

carbonate that could be within the chert matrix because of the high spatial resolution of the ion microprobe and the greatly enhanced ionization yield (factor ~100) for C^- from reduced carbon phases compared to that from carbonate minerals. Thus, not only do these data confirm the intrinsic isotopic variability of the previous TOC analyses, but also they demonstrate that this variability exists on the level of preserved carbon from individual microorganisms.

The isotopic compositions of the Bitter Springs microfossils range from $\delta^{13}C_{PDB}$ = –21‰ to –32‰. These values are consistent with carbon fixation via the Calvin cycle which produces a depletion in ^{13}C of ~20 to 35‰ (Roeske and O'Leary, 1984; Schidlowski et al., 1983; Sirevåg et al., 1977). Thus, the isotopic data corroborate the morphology-based assignment of the taxa analyzed to the crown group cyanobacteria (Schopf, 1968; Schopf and Blacic, 1971). However, in the absence of such morphologic evidence, the isotopic data could not be considered definitive because the degree of isotopic fractionation exhibited by differing metabolic pathways can overlap. For example, although the acetyl-CoA pathway can exhibit substantially greater fractionation than the Calvin Cycle (yielding products >40‰ depleted in ^{13}C relative to dissolved inorganic carbon), the two pathways often yield isotopically similar products (Fuchs et al., 1979; Preuβ et al., 1989; Schidlowski et al., 1983). On the other hand, both the reductive TCA cycle and the 3-hydroxypropionate cycle do not produce high fractionation (<20‰ relative to dissolved inorganic carbon) (House, 1999; Preuβ et al., 1989; Sirevåg et al., 1977).

TABLE 3. ION MICROPROBE-MEASURED CARBON ISOTOPIC COMPOSITION OF PETRIFIED PRECAMBRIAN MICROFOSSILS

Taxon	Specimen #	Diameter of Cells (μm)	$\delta^{13}C_{PDB}$ (‰)	Calculated Error ($1\sigma_m$)	Number of Analyses	MSWD
BITTER SPRINGS FORMATION:						
Myxococcoides	a	11.5	-21.0	1.7	5	0.1
	b*	7.5	-23.4	1.6	2	0.0
	c*	7.0	-27.0	1.3	2	0.2
	d	17.0	-28.8	1.4	7	0.4
Eomycetopsis	e	3.0	-23.2	1.1	6	3.8
	f	3.3	-24.0	1.1	6	3.0
	g*	3.0	-27.4	1.2	4	0.6
	h	3.0	-28.3	1.4	7	0.3
Glenobotrydion	i	7.5	-24.8	1.0	6	0.6
	j	12.5	-25.2	1.7	2	0.1
	k	7.0	-26.7	1.4	8	1.4
	l	8.0	-27.3	1.7	4	0.1
	m	12.5	-27.5	2.9	2	0.0
	n	9.5	-29.0	0.9	6	1.1
Cephalophytarion	o	2.5	-31.9	1.2	6	1.0
GUNFLINT FORMATION:						
Gunflintia	p*	3.2	-32.5	0.7	2	0.0
	q	3.5	-32.6	1.3	6	0.2
	r*	2.0	-35.7	1.8	2	0.0
	s*	2.2	-37.2	0.7	2	0.0
Huroniospora	t*	6.5	-31.7	0.5	2	26
	u	4.0	-34.6	1.5	4	0.3
	v*	10.5	-35.6	0.6	2	0.1
	w*	7.5	-35.7	0.8	2	1.0
	x*	9.5	-36.1	1.7	2	0.7
	y*	7.0	-36.0	1.7	2	0.1
	z	10.5	-36.5	1.4	6	0.9
	aa	8.5	-36.5	1.3	6	0.6
? Huroniospora	ab*	8.0	-33.9	0.5	2	2.0
	ac	13.5	-42.7	1.1	6	0.7
	ad	10.0	-45.8	1.2	6	0.5

Note: PPRG 215-1 was used as a standard for these measurements (see text). Fossils are from stromatolitic cherts of the ~850-Ma-old Bitter Springs Formation of central Australia (a though o) and ~2,100-Ma-old Gunflint Formation of southern Canada (p through ad). The analytical uncertainty of each individual microfossil analysis was based on the internal precision of the analysis adjusted to reflect the reproducibility of analyses of the standard. The reported $\delta^{13}C$ compositions are the weighted mean of multiple individual analyses ±1 standard error of the mean ($1\sigma_m$).

*Specimens analyzed using multicollection mode.

The isotopic compositions of the Bitter Springs microfossils vary by several parts per thousand among members of each taxon, and the $\delta^{13}C_{PDB}$ values of specimens within a single thin section differ by as much as ~10‰. This spread in $\delta^{13}C_{PDB}$ values demonstrates that isotopic compositions were not homogenized during diagenesis and subsequent organic maturation. Similarly, because the carbon isotopic compositions of well-preserved microfossils overlap both the TOC measurements and ion microprobe analysis of a spot containing finely particulate (nonmicrofossil) kerogen diffused in the chert ($\delta^{13}C_{PDB}$ = –25.2 ± 1.8‰), it is unlikely that the carbon isotopic compositions of the cells were altered substantially during the initial fossilization process relative to the diffuse organic matter within the chert. Thus, the observed heterogeneity appears to reflect original isotopic differences, probably due to either vital processes or to local differences in the composition of carbon available for biologic fixation.

The Gunflint microfossils analyzed are more ^{13}C depleted and isotopically more variable than those of the Bitter Springs, spanning a range of $\delta^{13}C_{PDB}$ values from –32‰ to –45‰ (Table 3, p through ad). Seven well-preserved specimens of *Huroniospora* (Table 3, u through aa, and Fig. 2F), a unicellular taxon of uncertain but possibly cyanobacterial affinity (Barghoorn and Tyler, 1965), have $\delta^{13}C_{PDB}$ values that cluster at about –36‰, differing appreciably from two highly degraded *Huroniospora*-like specimens (Table 3, ac and ad, and Fig. 2G) which have extremely ^{13}C-depleted values of –42‰ ± 1.1‰ and –45‰ ± 1.2‰. These extremely ^{13}C-depleted specimens are not consistent with carbon fixation via the Calvin cycle (Schidlowski et al., 1983) unless the source carbon was already fractionated, derived, for example, from the microbial degradation of

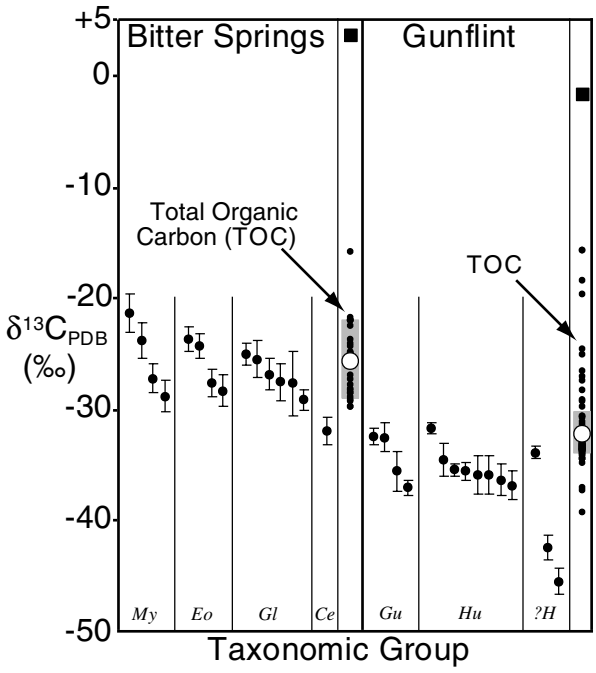

Figure 3. Ion microprobe-measured carbon isotopic compositions of microfossils listed in Table 3, compared with values reported for total organic carbon (TOC) and carbonate carbon (Hoering, 1961; Schidlowski et al., 1983) (squares) from cherts of each formation. For each measurement, narrow vertical bar indicates calculated error (1 σ_m). For TOC values (Bitter Springs: *n* = 30, including one sample of isolated kerogen; Gunflint: *n* = 76, including five kerogens), shaded bars show ranges of 80% of values closest to means, denoted by open circles (Strauss and Moore, 1992). Taxa analyzed (Barghoorn and Tyler, 1965; Schopf, 1968; Schopf and Blacic, 1971) include *Myxococcoides* (*My*), *Eomycetopsis* (*Eo*), *Glenobotrydion* (*Gl*), *Cephalophytarion* (*Ce*), *Gunflintia* (*Gu*), *Huroniospora* (*Hu*), *?Huroniospora* (*?H*).

other organic materials in the fossil microbial mat. These atypical values do not seem to be the result of secondary, diagenetic alteration because specimen ab, a similarly highly degraded *Huroniospora*-like specimen, is not atypically depleted in ^{13}C. Broadly, the Gunflint microfossils are more ^{13}C-depleted than the Bitter Springs microfossils, a composition that follows closely the trend of whole-rock carbon isotopic results typical of this time interval (Strauss and Moore, 1992).

CONCLUSIONS

We have established that carbon isotopic analyses of individual microscopic fossils can be performed with high (~1‰ to 2‰) precision and accuracy, and that ion microprobe analyses of Precambrian microorganisms are capable of detecting isotopic differences of the magnitude expected from diverse pathways of autotrophic carbon fixation. With the exception of the particularly ^{13}C-depleted degraded unicells from the Gunflint Formation, all microfossils analyzed have $\delta^{13}C_{PDB}$ values consistent with carbon fixation via the Calvin cycle and, thus, with earlier studies that assigned them to the cyanobacteria on the basis of morphology (Barghoorn and Tyler, 1965; Schopf, 1968; Schopf and Blacic, 1971).

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