Despite evidence for liquid water at the surface of Mars during the Noachian epoch, the temperature of early aqueous environments has been impossible to establish, raising questions of whether the surface of Mars was ever warmer than today. We address this problem by determining the precipitation temperature of secondary carbonate minerals preserved in the oldest known sample of Mars’ crust—the approximately 4.1 billion-year-old meteorite Allan Hills 84001 (ALH84001). The formation environment of these carbonates, which are constrained to be slightly younger than the crystallization age of the rock (i.e., 3.9 to 4.0 billion years), has been poorly understood, hindering insight into the hydrologic and carbon cycles of earliest Mars. Using “clumped” isotope thermometry we find that the carbonates in ALH84001 precipitated at a temperature of approximately 18 °C with water and carbon dioxide derived from the ancient Martian atmosphere. Furthermore, covarying carbonate carbon and oxygen isotope ratios are constrained to have formed at constant, low temperatures, pointing to deposition from a gradually evaporating, subsurface water body—likely a shallow aquifer (meters to tens of meters below the surface). Despite the mild temperatures, the apparently ephemeral nature of water in this environment leaves open the question of its habitability.

The extreme difficulty in achieving mild surface temperatures in early Mars climate models (1) is in disagreement with widespread geomorphological evidence for surface water runoff during the Noachian epoch (2, 3). A robust determination of the temperature at or near the surface of Noachian Mars would provide insight into this apparent paradox, but has been challenging to establish. Carbonate minerals can record and preserve information regarding the temperature and chemistry of their formation environment through aspects of their oxygen and carbon isotopic composition (δ18O and δ13C, respectively). These minerals have been observed in a range of Martian geological materials, including dust, bedrock outcrops, and several meteorites (4–9). Collectively, these observations highlight a role for carbonate formation in the global Martian carbon cycle and the evolution of its atmosphere. The oldest known carbonate from Mars, or any other planetary body, occurs as a minor constituent (approximately 1% by weight) in the meteorite Allan Hills 84001 (ALH84001), which has a crystallization age of approximately 4.1 billion years (10). The carbonate in ALH84001, geochronologically constrained to be slightly younger in age (between 3.9 and 4.0 billion years old; (11)), provides a unique window into the hydrologic cycle, carbon cycle, and climate of Noachian Mars.

Texturally, the carbonates in ALH84001 occur as (i) chemically and isotopically zoned ovoid concretions, veins, and void fillings and (ii) regions of massive carbonate, variably intergrown with unweathered feldspathic glass and orthopyroxene, the latter of which makes up the bulk of the meteorite (12–14). The concretions display wide systematic variation in chemical and isotopic composition. They have Ca-Fe-rich cores with δ18OSMOW (O isotope ratios reported relative to Standard Mean Ocean Water) values as low as approximately 5‰ and δ13CDB (C isotope ratios reported relative to the Pee Dee Belemnite standard) values of 25–35‰ (15–18). Their rims are Mg-rich, sometimes nearly pure MgCO3, and have δ18OSMOW and δ13CDB values as high as approximately 30‰ and 65‰, respectively. The veins and void fillings display covarying chemical and isotopic compositions similar to the concretions (12). The massive carbonates are usually internally homogeneous, though chemical and isotopic variability has been observed between isolated occurrences. Several studies have reported an ankerite (Ca-rich) and isotopically light (δ18OSMOW of 0–10‰) composition for the massive carbonates (e.g., ref. 16).

The carbonates in ALH84001 have been studied by a variety of geochemical and petrographic methods, but no agreement exists concerning the processes and environment of their precipitation. Hypotheses for their formation span a range of temperatures between subfreezing and more than 700 °C, a range of depths between the surface and several kilometers, and a range of processes including impact melting of preexisting carbonates, hydrothermal alteration of crustal rocks, precipitation from a rapidly evaporating water body, and biological activity (13–16, 19–28). Textural observations indicate that after carbonate growth, ALH84001 experienced brief, localized, shock-related heating, but natural remanent magnetization and argon thermochronometry place upper limits of approximately 40 °C on the short-duration (approximately 10 min) and approximately 20–30 °C on the long-duration (billions of years) thermal history of this rock (29, 30).

Previous methods for estimating the temperature of carbonate formation in ALH84001, though informative, have placed only loose constraints (e.g., refs. 29 and 30) or have depended on assumptions about the chemical and isotopic composition of the carbonate’s parent fluid (e.g., refs. 16 and 22). Multiply substituted, or “clumped,” isotope thermometry avoids this problem because it is based on homogeneous thermodynamic equilibrium within the carbonate mineral (31, 32). The abundance of rare isotopologues, measured as mass 47CO isotopes, has increased from acid digestion of carbonates minerals, is higher than predicted from a random distribution of the heavy isotopes. This excess is denoted Δ47 and varies systematically with carbonate precipitation temperature (32, 33). With temperature known, measurements of the δ18O and δ13C of the carbonates provide direct constraints on the isotopic compositions of their parent fluid (water and CO2).

Author contributions: I.H. and J.M.E. designed research; I.H. performed research; I.H. contributed new reagents/analytic tools; I.H., W.W.F., and J.M.E. analyzed data; and I.H., W.W.F., and J.M.E. wrote the paper.
The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

1To whom correspondence may be addressed at: Environmental Sciences and Energy Research, Weizmann Institute of Science, Rehovot, 76100, Israel. E-mail: Itay.Haley@Weizmann.ac.il.

2Present address: Weizmann Institute of Science, Rehovot, 76100, Israel. This article contains supporting information online at www.pnas.orglookup/suppl/doi:10.1073/pnas.1109444108/-/DCSupplemental.
Table 1. Isotopic composition of ALH84001 carbonates extracted by stepped acid digestion

<table>
<thead>
<tr>
<th></th>
<th>0–1 h</th>
<th>1–4 h</th>
<th>4–12 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>δ^{18}O‰</td>
<td>δ^{13}C‰</td>
</tr>
<tr>
<td>Aliquot A</td>
<td>—</td>
<td>16.85</td>
<td>40.87</td>
</tr>
<tr>
<td>(320 mg)</td>
<td>—</td>
<td>13.05</td>
<td>35.52</td>
</tr>
<tr>
<td>Aliquot B</td>
<td>5</td>
<td>13.38</td>
<td>34.60</td>
</tr>
<tr>
<td>(1,625 mg)</td>
<td>(±0.28)</td>
<td>(±0.07)</td>
<td>(±0.039)</td>
</tr>
<tr>
<td>Aliquot C</td>
<td>8</td>
<td>14.11</td>
<td>35.14</td>
</tr>
<tr>
<td>(1,550 mg)</td>
<td>(±0.16)</td>
<td>(±0.02)</td>
<td>(±0.027)</td>
</tr>
</tbody>
</table>

ΔO and ΔC are reported relative to the SMOW and PDN standards, respectively. N is the number of data acquisitions suitable for clumped isotope analysis (typical clumped isotope measurements comprise eight acquisitions), reflecting the gas yield from the digestion steps. Error estimates are based on identical treatment of carbonate standards of known bulk and clumped isotopic composition, which was only done for aliquots B and C (SI Text).

Results

We performed stepped phosphoric acid digestion of three aliquots of ALH84001 and measured the bulk and clumped isotopic composition of the evolved CO₂ (see Methods and SI Text). The amount of CO₂ evolved from the digestion steps was about one-tenth to one-third the amount typically required for clumped isotope analysis. Additionally, the acid digestion duration was up to 36 times longer than typically applied, increasing the potential for contamination. This combination of factors necessitated special extraction apparatus, purification steps, and analytical protocols (SI Text, Table S1, and Figs. S1–S3). The Δ^{18}O and Δ^{13}C values of the carbonate extracted in the three digestion steps (Table 1 and Table S2) were reproducible among the three different meteorite aliquots and fall within the range of previous measurements (15–18, 21, 22). Combining these Δ^{18}O and Δ^{13}C measurements with prior constraints on the relationship between the stable isotope and major element composition in ALH84001 carbonates (15–18), we estimated the chemical composition of carbonate sampled by the digestion steps (Fig. 1 and SI Text). The sequential extraction technique yielded increasing Mg content with reaction time, a relationship expected from the slower dissolution of the Mg-rich outer concretion layers (34).

The first aliquot of the meteorite (approximately 320 mg) yielded too little gas for reliable Δα_{47} measurements, but has Δ^{18}O and Δ^{13}C values that are consistent with subsequent extractions of two larger aliquots (1,625 and 1,550 mg, respectively). The Δα_{47} measurements of these two larger aliquots (Table 1) yield six temperatures between 31° and 30°C, all within uncertainty of an error-weighted average temperature of 18 ± 4°C.

Discussion

Establishing the Robustness of the Temperature Determination.

We addressed potential sources of uncertainty (outside of analytical uncertainty) in interpreting the Δα_{47} data as a precipitation temperature for the carbonates in ALH84001. The crystal orientation distribution of the carbonate concretions (SI Text and Fig. S4) suggests that the carbonates have not been subject to recrystallization since their radial growth from aqueous solution. In the absence of recrystallization, resetting of the carbonates’ isotopic composition to yield the observed, strongly covarying relationship between Δ^{18}O and Δ^{13}C is unlikely. Together, these observations indicate that the measured Δα_{47} was acquired during initial carbonate precipitation.

Precipitation at high temperature and subsequent reordering of carbonate clumped isotopes upon cooling is unlikely because the temperature recorded under such a scenario (i.e., blocking temperature) would be approximately 200–300°C (35, 36). Mixing between two physical or isotopic end members with bulk isotopic composition at either end of the observed Δ^{13}C and Δ^{18}O range in ALH84001 carbonate may lead to spuriously low apparent temperatures, but is also unlikely, as indicated both by previous observations of the distribution of ALH84001 carbonate chemical and isotopic composition (15–18) and by limits on the time scale for isotopic equilibrium (SI Text and Fig. S5).

Several additional hypotheses for interpreting the Δα_{47} data can be rejected on the basis of the relationships observed among the Δ^{18}O, Δ^{13}C, and Δα_{47} measured in the carbonate (Fig. 2). Carbonate precipitation over a range of temperatures (e.g., ref. 22) does not fit the observed δ^{18}O and δ^{13}C array (Fig. 2A). Because the stepped acid digestion could not perfectly separate the concretion cores from their rims, the range of δ^{18}O_{SMOW}, we observed was narrow (approximately 13–19‰) relative to the full range observed in microprobe studies (approximately 5–30‰). As a result, the uncertainty in a linear fit to the reliable δ^{18}O–Δα_{47} data (aliquots B and C) is large at the ends of the full δ^{18}O range (Fig. 2B, dashed curves). Thus, in a formal hypothesis testing sense, the 95% confidence bounds marginally include an explanation of the data by kinetic isotope effects (KIEs) of the sort observed in some cave and cryogenic carbonates (37–40). These KIEs, which are thought to arise from rapid, disequilibrium CO₂ degassing, have been observed to lead to spurious apparent values of Δα_{47} that are lower than the true values by as much as 0.12‰. Applied to our measured Δα_{47}, this would translate into an error-weighted average temperature of carbonate formation in ALH84001 of ~7°C (instead of approximately 18°C if the Δα_{47} we measure is correct). Temperatures below 0°C can be rejected because they would cause the water from which the carbonates formed to freeze and are thus inconsistent with the increase in δ^{18}O from the concretion cores to their rims. The remaining temperature range between 0 and 18°C is possible, but the large uncertainty in the fit and the better correspondence to a hypothesis of constant temperature (Fig. 2B) lead us to conclude that KIEs of the type discussed above are unlikely in

Fig. 1. Major element composition of the ALH84001 carbonates digested in the three steps, estimated from their δ^{18}O and δ^{13}C values and correlations between major element and isotopic composition (see text).

Fig. 1. Major element composition of the ALH84001 carbonates digested in the three steps, estimated from their δ^{18}O and δ^{13}C values and correlations between major element and isotopic composition (see text).
predictions for the relationship between constant temperature. The temperature of 21.2 °C is the average temperature of the two leftmost data points. The light and dark orange wedges are model environment and mechanism.

in the carbonates to develop a model for their formation constraining temperature, we are able to use the isotopic variation in the carbonates to develop a model for their formation.

For the Δ13C constraints, we find that the striking core to rim coevolution of δ18O_Smow (approximately 5 → 30‰) and δ13C_PDB (approximately 25 → 65‰), observed in previous ion-microprobe studies (15–18) is best explained by gradual evaporation and CO2 degassing under equilibrium conditions. The observed isotopic variation in the carbonates suggests a system in poor short-term communication with the atmosphere—otherwise the δ18O and δ13C would be buffered by exchange with the atmospheric CO2 reservoir. This rules out, for example, formation of the ALH84001 carbonates in a long-lasting, open lake. Instead we suggest that distillative loss of the isotopically light vapor phase (gaseous CO2 and water vapor) from a semiisolated environment (Fig. 3A) gradually enriched the residual water and dissolved inorganic carbon in the heavy isotopes, resulting in the coupled increase in δ18O and δ13C values from the concretion cores to their rims. A system open to the atmosphere, where evaporation and carbonate precipitation occurred too rapidly to allow equilibrium with the gas phase, may also be consistent with the

the case of ALH84001 carbonates. Altogether the data are best explained by carbonate precipitation at a constant temperature of approximately 18 °C. This result rules out a high-temperature origin of ALH84001 carbonates (e.g., refs. 13, 14, and 19) and imposes strict constraints on the remaining low-temperature hypotheses.

Fig. 2. Comparison of Δ47, δ18O, and δ13C values (see SI Text) with models for the isotopic variation in ALH84001 carbonates. (A) Relationship between δ13C and δ18O. The solid black line and the broken black curves are a weighted total least squares linear fit to the data and a 95% confidence bound on the fit, respectively. The diagonal gray line shows the equilibrium relationship between δ13C and δ18O over the range of temperatures required to explain the range of δ18O_Smow observed in microprobe studies (approximately 5–30‰). A hypothesis of evolving temperature can be rejected on the basis of the misfit between these lines. (B) Relationship between Δ47 and δ18O. The solid black line and the broken black curves are a weighted total least squares linear fit to the data and a 95% confidence bound on the fit, respectively. The diagonal gray line shows the equilibrium relationship between Δ47 and δ18O over the range of temperatures required to explain the observed δ18O_Smow range (approximately 5–30‰). The horizontal gray line shows the isotopic evolution due to evaporation at a constant temperature. The temperature of 21.2 °C is the average temperature of the two leftmost data points. The light and dark orange wedges are model predictions for the relationship between Δ47 and δ18O that is expected from rapid degassing and associated kinetic isotope effects (38). The confidence bounds on the fit to the data are most consistent with constant temperature, less consistent with an evolving temperature (which can be ruled out by the relationship between δ13C and δ18O) and only marginally include kinetic isotope effects due to rapid degassing (see text).

Formation Environment of ALH84001 Carbonates. Previously only loose constraints on the temperature of carbonate growth in ALH84001 were available, and past models for their formation required one or more assumptions about temperature, the open/closed nature of the system, the ratio of CO2 to H2O, or the isotopic composition of the parent fluid. Here, by independently constraining temperature, we are able to use the isotopic variation in the carbonates to develop a model for their formation environment and mechanism.

From the Δ47 constraints, we find that the striking core to rim coevolution of δ18O_Smow (approximately 5 → 30‰) and
Conclusions

In sum, the evidence points to a short-lived, low-temperature, aqueous environment, initially containing surface-derived CO$_2$ and H$_2$O but subsequently characterized by poor communication with the atmosphere, such that isotopic buffering did not occur. Distillative loss of CO$_2$ and H$_2$O can explain the observed $\delta^{18}O$ and $\delta^{13}C$ trends. This combination of characteristics is consistent with partial to complete evaporation of a subsurface aqueous reservoir, coupled to carbonate precipitation and CO$_2$ degassing under equilibrium conditions. We imagine that an ephemeral regolith aquifer at a depth of a few to tens of meters would be a plausible setting for this process (Fig. 3). The existence of a near-surface aqueous environment during the Noachian epoch, recorded by the carbonates in ALH84001, is consistent with, though need not require, a warmer early Mars—a hydrothermal or impact-related source for the required heat is equally possible. Though the mild temperatures point to an environment that might be considered habitable, the presence of water was also ephemeral, suggesting a time frame probably too short for life to have evolved de novo.

Methods

Three aliquots of ALH84001 were subjected to stepped phosphoric acid digestion. Because of the slower dissolution kinetics of magnesian carbonates, the first step in each of the digestions (0–1 h) sampled the faster-reacting Ca-rich carbonates, whereas the second and third steps (1–4 and 4–12 h, respectively) sampled more Mg-rich carbonates (34). Finer separation (e.g., by lower reaction temperature or shorter reaction intervals) was not attempted due to the relatively large amount of CO$_2$ required for clumped isotope analysis, the susceptibility of small samples to contamin-
carbon removed by carbonate precipitation is equal to the loss of the precipitating cation, which is solved by the model. The remaining carbon lost from solution is divided between transport to the surface and an increase in pore pCO2. The carbon isotopic evolution, therefore, depends on the relative proportions of these removal processes (carbonate precipitation, diffusive transport, and pore overpressurization). See SI Text for a full description of the model and parametric sensitivity analysis results.

ACKNOWLEDGMENTS. We thank N. Kitchen for assistance with the isotopic analyses. We thank two anonymous reviewers for valuable comments that have improved this work. I.H. was funded by a Texaco Postdoctoral Fellowship (Division of Geological and Planetary Sciences, Caltech). J.M.E. acknowledges funding from National Aeronautics and Space Administration and National Science Foundation-Division of Earth Sciences.

Supporting Information

Halevy et al. 10.1073/pnas.1109444108

SI Methods

1. Extraction and Purification. We performed three stepped phosphoric acid digestions of gently crushed bulk Allan Hills 84001 (ALH84001) at a temperature of 90±1 °C, each consisting of three digestion steps. The aliquot weights were approximately 320, 1,625, and 1,550 mg (henceforth A, B, and C, respectively). The samples were loaded into glass boats, placed in the experimental apparatus (Fig. S1) and pumped down under vacuum for more than 16 h before being dropped into the acid. The CO2 evolved from acid digestion was continuously trapped in a glass U trap immersed in liquid N2, passing first through a coiled glass trap immersed in an ethanol–dry ice slush (approximately −75 °C) to remove water.

When a reaction step was complete (after 1, 4, and 12 h), the gas in the U trap was pumped over to remove noncondensable gases, passed through a coiled trap immersed in a pentane slush (approximately −125 °C) to remove trace contaminants (SO2 and some organic molecules), and frozen into a valve sample vial immersed in liquid N2. The sample vials were immediately taken to analysis. The analyze CO2 was carried on a helium flow through 0.3 m of ½" outer diameter quartz tube packed with silver wool to remove H2S and then through 2.5 m of ⅛" outer diameter stainless steel column packed with Supelco Porapak-Q™ (porous polymer adsorbent) and held at −20 °C to remove water and miscellaneous contaminants. The cleaning steps described above were tested on standards of known bulk and clumped isotopic composition and found not to affect the measured isotopic values.

2. Microvolume Measurements, Standardization, and Error Estimates. The isotopic composition of CO2 was measured on a Thermo Finnigan MAT 253 gas source isotope ratio mass spectrometer configured to measure masses 44 through 49 (1). The amounts of evolved gas were too small for reliable clumped isotope measurements in dual-inlet mode. To overcome this we installed two nearly identical 10-cm long electroformed nickel tubes with an outer diameter of 0.06125" and an inner diameter of 0.02" (henceforth microvolume) on the sample and reference side (Fig. S2A). The tubing material was chosen to minimize surface area and reactivity. The sample gas was frozen into the sample-side microvolume by immersion in liquid N2 and subsequently thawed in the microvolume alone (excluding the bellows or any other tubing). The pressure in the microvolume allowed several reliable clumped isotope data acquisitions before the gas ran out. A similar pressure was reached in the reference-side microvolume by compressing the reference-side bellows and closing off the reference-side microvolume from the bellows. The reference-side bellows were adjusted after every extraction to match the pressure of the gas remaining in the sample-side microvolume. The pressure on the sample and reference side was, in general, balanced within 1.5% and mostly within <0.8%.

Microvolume measurements of standard materials of known bulk and clumped isotopic composition revealed a drift in the measured δ18O, δ13C and Δ47 relative to low-pressure dual-inlet measurements of the same materials (Fig. S2 B–D). The δ18O became progressively heavier in all experiments by as much as 2‰, whereas the δ13C became either heavier, lighter, or remained nearly constant (within measurement error). The drift in δ13C was at most a few tenths of a permil. The drift in δ18O and δ13C may be related to CO2 vapor pressure effects, which produce large oxygen isotope fractionations but only very modest carbon isotope fractionations (2). The sample CO2 was frozen into the microvolume and, once thawed, was free to flow through a capillary tube toward the ion source. As a result, the δ18O of the first CO2 to be measured was low relative to its bulk value and became higher during subsequent acquisitions, until stabilizing at a value slightly higher than the bulk value, due to the isotopically light material that was lost early in the measurement. The known δ18O and δ13C of the standard materials was retrieved by calculating an average of the δ18O and δ13C measured out of the microvolume, weighted by the average voltage (on the faraday cup measuring mass 44 CO2 ) of each acquisition (Fig. S2B). A small offset remained from the values measured in dual-inlet mode for both δ18O and δ13C (Table S1). After correction for this offset, the 95% confidence interval on the remaining error was ±0.281‰ and ±0.072‰ for δ18O and δ13C, respectively, for the period of extraction B and ±0.159‰ and ±0.024‰, respectively, for the period of extraction C. This provides an upper limit on the measurement error in δ18O and δ13C instead of a formal error estimate, which here tends to grossly overestimate the error. During the period of extraction A this standardization had not yet been implemented and so no empirical error estimates exist for this period.

The raw measured value of Δ47 was corrected to a “heated gas” reference frame, as established in previous clumped isotope studies (1). This correction is essentially a subtraction of a composition-dependent value from the raw Δ47. The correction is a linear function of δ17, even up to the very high values of Δ47 observed in ALH84001 (approximately 50‰; Fig. S3). After this correction, the value of Δ47 of small samples was found to be positively offset from the true known value. It is not known how clumped isotopeologues behave in CO2 ice/vapor systems, but this increase in Δ47 of small samples occurs for samples that had not been frozen into a microvolume, suggesting that an additional process may be involved. The notion that something other than measurement out of a microvolume is responsible for the offset is supported also by the observation that the weighted average of Δ47 measured out of a microvolume was always lower (closer to the true value) than the values measured at low pressure in dual-inlet mode. The reason for this offset may be related to scrambling in the ion source, a constant background ion flux, a sample size, and reaction duration-dependent contamination acquired during the extraction, or some combination of these and other factors. An extensive set of experiments on small amounts of standards of known bulk and clumped isotopic composition did not unambiguously reveal the source of the offset, but showed it to be predictable and correctable. In the weeks before and after the extraction of aliquots B and C we subjected carbonate standards of known Δ47 to exactly the same extraction, purification, and measurement procedures as the ALH84001 carbonates and recorded the offset from the true value (Table S1). The offsets were averaged and this average value subtracted from the values of Δ47 measured for the ALH84001 carbonates. The absolute value of the remaining error on the Δ47 of standards treated in the same way was averaged and two standard deviations were added to give a 95% confidence interval on the error remaining after such treatment. The Δ47 values reported for extractions B and C are in error from the true value by ±0.039‰ and ±0.027‰, respectively. As with the bulk isotopic composition, no empirical error estimates exist for Δ47 during the period of extraction A, as the standardization had not yet been applied.

A second error estimate for Δ47 was derived from the counting statistics limit to precision. For the first and second steps (0–1 and 1–4 h, respectively) in extractions B and C, this error is smaller than the error based on standards of known composition, de-
scribed above. For the third steps in these extractions (4–12 h), the counting statistics error is larger. In all cases the larger of the two error estimates is the one reported (Table S2). For extraction of aliquot A, only the counting statistics limit is reported, as the error estimation based on standards of known composition had not yet been developed and applied. In combination with the much smaller size of aliquot A (approximately 5–6 times smaller than aliquots B and C), the less well-developed extraction and purification procedure, and the absence of experience making microvolume measurements at that time, we have much more confidence in the results obtained from extraction of aliquots B and C. We focus on these in the main text, but report the values measured from extraction of aliquot A in Table S2.

3. Treatment of Anomalous Oxygen Isotope Composition of ALH84001 Carbonates. The carbonates in ALH84001 have mass-anomalous oxygen isotope compositions, with $\delta^{18}$O of approximately 0.8‰ (3). To account for this, we used the raw ratios of mass 45 and 46 CO$_2$ to mass 44 CO$_2$ ($^{46}$R and $^{48}$R, respectively) to solve the following three equations for the samples’ isotopic ratios $^{13}$R, $^{17}$R, and $^{18}$R ($^{13}$C/$^{12}$C, $^{17}$O/$^{16}$O, and $^{18}$O/$^{16}$O, respectively):

$$45\ R = \frac{^{13}\ R + 2^{17}\ R}{\text{(S1)}}$$

$$46\ R = 2^{18}\ R + 2^{13}\ R + 17^{17}\ R,$$  

$$17^{17}\ R = K_1^{17}\ R^4 + K_2,$$  

where $K_1 = 17^{17}\ RVSMOW / (19^{18}\ RVSMOW)^4$, $K_2 = 17^{17}\ RVSMOW \times \Delta^{17}/1,000$, $\Delta$ is the slope of the oxygen isotope mass-fractionation line, and $^{18}$RVSMOW is the ratio $^{15}$O/$^{16}$O in VSMOW (Vienna Standard Mean Ocean Water). These values of $^{13}$R, $^{17}$R, and $^{18}$R were then used to calculate the random distribution of the heavy isotopes, which was used in the calculation of $\Delta_{47}$. With a $\Delta^{18}$O of 0.8‰, the $\Delta_{47}$ increased by approximately 0.035‰, corresponding to a temperature decrease of approximately 7°C (relative to the same calculation carried out with $\Delta^{17}$O of 0‰). The samples’ $\delta^{18}$O and $\delta^{17}$C typically increased by approximately 0.027‰ and approximately 0.24‰, respectively.

4. Major Element Composition Estimates. The microprobe $\delta^{18}$O and $\delta^{17}$C data (4–7) were correlated by Mg content in six bins of Mg normalized mole fraction ($X_{Mg}$), each with a width of 0.1. The average and two standard deviations of the $\delta^{18}$O and $\delta^{17}$C in each of the six bins are shown in Fig. 4 in the main text (gray crosses). A weighted least squares linear fit with 95% confidence bounds is also shown in Fig. 4. We used this fit, together with the $\delta^{18}$O—major element composition data to generate a synthetic dataset consisting of 105 points, as follows: Combinations of $\delta^{18}$O-$X_{Mg}$-$X_{Fe}$-$X_{Ca}$, were picked at random from a pool of 109 ion–microprobe measurements (4–6). A normally distributed random component of up to ±20% of the microprobe values was added to all four values. A value of $\delta^{18}$C was generated using the fit described above, and a normally distributed random component of ±20% was added to its value. The synthetic data were then used to calculate a probable combination of $X_{Mg}$, $X_{Fe}$, $X_{Ca}$, given the $\delta^{18}$O and $\delta^{17}$C measured in our bulk carbonate acid digestion (Fig. 1 in the main text).

5. Isotopic Evolution Model. The observed carbon, oxygen, and $\Delta_{47}$ data are most consistent with precipitation at near-constant temperature (see main text, Fig. 2, and SI Text section Reliability of Temperature Determination). Additionally, anomalous oxygen and sulfur isotope signatures in ALH84001 indicate a surface source for the fluids from which the carbonates (and sulfides) formed, but the evolution of $\delta^{17}$C implies poor communication with the atmosphere during carbonate precipitation (see main text). We used these observations to develop a quantitative model of carbonate precipitation in ALH84001.

For reasons of carbonate chemistry, a solution sourced at the surface is likely to have a ratio of $H_2O$ to CO$_2$ exceeding $10^3$. For example, even if the ancient Martian atmosphere contained 1 bar of CO$_2$, a solution with no dissolved cations (i.e., where the partial pressure of CO$_2$ alone controlled the solution pH) would have a water to CO$_2$ ratio of approximately 1,430 and a pH of approximately 3.9 at a temperature of 20°C. A solution saturated with a carbonate mineral (i.e., containing dissolved cations) would have a higher pH, would be able to hold a higher concentration of dissolved inorganic carbon (DIC), and would, therefore, have a lower ratio of water to CO$_2$. For example, saturation with siderite (FeCO$_3$) at pCO$_2$ of 1 bar requires 1.7×$10^{-3}$ mol per liter of Fe$^{2+}$. This concentration of dissolved ferrous iron would increase the pH only to approximately 5.0 and decrease the water to CO$_2$ ratio to approximately 1,570 (from a value of 1,430 with no dissolved cations). Saturation with calcite, which is more soluble, would increase the pH to approximately 5.9 and decrease the water to CO$_2$ ratio to approximately 1,060. Thus, the $\delta^{18}$O of carbonates precipitated from an aqueous solution formed at the surface of early Mars would be water-buffered. Under equilibrium conditions, the carbonates’ $\delta^{18}$O would be controlled by the processes affecting the $\delta^{18}$O of the water, rather than by processes that fractionate the oxygen isotopes between species that comprise the DIC pool.

In the scenario described in the main text and shown in Fig. 3, a subsurface aqueous solution evaporates. The evaporation drives carbonate precipitation and CO$_2$ degassing. The distillative loss of water to evaporation and CO$_2$ to degassing cause an increase in the $\delta^{18}$O and $\delta^{13}$C of the residual fluid, respectively. In this system, the total dissolved inorganic carbon, DIC (in moles), can be expressed in two ways:

$$\text{DIC} = pCO_2 \times KH \times \left(1 + K_{A1} \frac{[H^+]}{[H^+]^2}\right) \times H_2O.$$  

$$\text{DIC} = \frac{K_{SP}}{M^{2+}/H_2O} \times \left(1 + \frac{[H^+]}{[K_{A1}]} + \frac{[H^+]^2}{[K_{A1}]}\right) \times H_2O.$$  

Eq. S4 describes equilibrium between the gas in the soil or rock pore space and the carbon in aqueous solution; Eq. S5 describes saturation of a carbonate mineral, MCO$_3$, $K_{A1}$, $K_{A2}$, and $K_{SP}$ are Henry’s Law constant for CO$_2$, the first and second carbonic acid dissociation constants, and the solubility product constant for MCO$_3$, respectively (SI Text section References for model constants). $[H^+]$ is the concentration of the hydronium ion (in moles per liter), $M^{2+}$ is the amount of the precipitating cation (in moles), $H_2O$ is the amount of water (in liters), and pCO$_2$ is the pore CO$_2$ pressure (in atmospheres).

Equating these two expressions for DIC, we obtain

$$pCO_2 \times KH \times K_{A1} \times K_{A2} \times \frac{M^{2+}}{[H^+]^2} = \frac{K_{SP}}{H_2O}.$$  

Charge balance dictates

$$[H^+] - [OH^-] - [HCO_3^-] - 2 \times [CO_3^{2-}] + 2 \times \frac{M^{2+}}{H_2O} + A = 0,$$  

and, given carbonate species equilibrium, can be written as

$$[H^+] - KH \times \frac{pCO_2 \times KH \times K_{A1}}{[H^+]^2} - 2 \times pCO_2 \times KH \times K_{A1} \times K_{A2} \times \frac{M^{2+}}{H_2O} + A = 0.$$
Here $K_W$ is the dissociation constant of water and $A$ is the total charge of nonprecipitating, conservative ions (in equivalents per liter). Rearranging Eq. S6 to express $M^{2+}$ as a function of $[H^+]$ and substituting this into Eq. S8 yields a quartic equation for $[H^+]$, which has only one real, positive root and can be solved given $pCO_2$, $A$, and $H_2O$. After solving for $[H^+]$ this value can be used in Eq. S6 to obtain the value of $M^{2+}$ and in Eq. S4 or S5 to obtain the value of DIC.

Prescribing initial values for $pCO_2$, $A$, and $H_2O$ and then progressively decreasing the amount of water to simulate an evaporating aqueous reservoir allows for all of the properties of the system to be solved as the water evaporates. As an amount of water, $\Delta H_2O$, is lost,

$$H_2O = H_2O_{i-1} - \Delta H_2O,$$  \[S9\]

$$\Delta M^{2+} = M^{2+}_{i-1} - M^{2+},$$  \[S10\]

where $M^{2+}_{i-1}$ and $M^{2+}$ are the amount of precipitating cation solved for with $H_2O_{i-1}$ and $H_2O$ liters of water, respectively. The total DIC loss is then

$$\Delta DIC = DIC_{i-1} - DIC = \Delta DIC_{carb} + \Delta DIC_{dgas} + \Delta DIC_{pres},$$  \[S11\]

where DIC$_{carb}$ and DIC$_{pres}$ are the total DIC solved for with $H_2O_{i-1}$ and $H_2O$ liters of water, respectively. The subscripts carb, dgas, and pres denote, respectively, DIC loss to carbonate precipitation, distillative CO$_2$ transport to the surface, and an increase in pore $pCO_2$. The amount of carbon lost to carbonate precipitation is equal to the amount of precipitating cation lost (Eq. S12), and the rest is divided between distillative CO$_2$ loss and pore pressure increase,

$$\Delta DIC_{carb} = \Delta M^{2+},$$  \[S12\]

$$\Delta DIC_{dgas} = f_{dgas} \times (\Delta DIC - \Delta DIC_{carb}),$$  \[S13\]

$$\Delta DIC_{pres} = (1 - f_{dgas}) \times (\Delta DIC - \Delta DIC_{carb}).$$  \[S14\]

Here $f_{dgas}$ is a modulating factor that defines how much of the carbon loss in excess of carbonate precipitation is transported toward the surface as opposed to increasing the pressure in the pores. The pore $pCO_2$ then evolves as

$$pCO_2 = pCO_2_{i-1} + \frac{\Delta DIC_{pres} \times N_A \times k_B \times T}{V},$$  \[S15\]

where $N_A$ is Avogadro’s constant, $k_B$ is Boltzmann’s constant, $T$ is the temperature, and $V$ the pore space volume not occupied by aqueous solution, which increases as the water evaporates. If $f_{dgas}$ is equal to 1, then there is no change in pressure, pH, and precipitating ion concentration (although the total amount of precipitating ion decreases as the solution loses water). If $f_{dgas}$ is less than 1, then the pore pressure increases, pH decreases, and the concentration of precipitating cation at carbonate mineral saturation increases (though its total amount progressively decreases). As mentioned in the main text, we find that a value of $f_{dgas}$ of approximately 0.9 best accounts for the coevolution of carbon and oxygen isotopic composition, the systematics of which are described below. That is, approximately 90% of the degassed CO$_2$ is transported to the surface and approximately 10% goes to increase the pore CO$_2$ pressure.

In a completely confined system, where water initially occupies all of the available pore space, the $pCO_2$ will increase until the solution becomes undersaturated with the carbonate mineral, at which time precipitation will cease. In a system that remains saturated (or supersaturated) and gradually loses CO$_2$ from pores that are overpressured with respect to the atmosphere, such as the environment we describe here, it is necessary only to have enough initial water-free pore space so as not to reach geologically unreasonable pressures. Even if we define the water in this system to occupy 90% of the initial available pore space, the resulting pressure increase for the required value of $f_{dgas}$ is at most 20% of the original pressure—a very modest value. Though it might be reasonable to consider a pore space nearly completely saturated with water and an increase in pressure greater than 20%, prescribing the water to occupy more than 90% of the available pore space results in numeric instability of the model.

The processes that remove water and DIC from the system described above also fractionate the isotopes of oxygen and carbon, resulting in coevolution of the isotopic composition. The relationship observed among the carbonate $\delta^{18}O$, $\delta^{13}C$, and $\Delta_{at}$ indicates isotopic equilibrium between the water and DIC. Therefore, we reequilibrate the oxygen isotopic composition between the water and DIC at every model step. The preequilibration isotopic composition of the water and DIC reservoirs is

$$^{18}R_{i}^{H_2O} \times H_2O = ^{18}R_{i-1}^{H_2O} \times H_2O_{i-1} - \Delta H_2O \times ^{18}R_{\text{evap}},$$  \[S16\]

$$^{18}R_{i}^{DIC} \times DIC = ^{18}R_{i-1}^{DIC} \times DIC_{i-1} - \Delta DIC_{carb} \times ^{18}R_{\text{carb}} - \Delta DIC_{dgas} \times ^{18}R_{\text{gas}} - \Delta DIC_{pres} \times ^{18}R_{\text{pres}},$$  \[S17\]

$$^{13}R_{i}^{DIC} \times DIC = ^{13}R_{i-1}^{DIC} \times DIC_{i-1} - \Delta DIC_{carb} \times ^{13}R_{\text{carb}} - \Delta DIC_{dgas} \times ^{13}R_{\text{gas}} - \Delta DIC_{pres} \times ^{13}R_{\text{pres}},$$  \[S18\]

where $^{18}R_{i}$ is the ratio of $^{18}O$ to $^{16}O$ in reservoir $x$ at step $i$ and $^{13}R_{i}$ is the ratio of $^{13}C$ to $^{12}C$ in reservoir $x$ at step $i$. The isotope ratios associated with the various loss processes depend on the fractionation factor ($\alpha$) between the water and DIC pools and the mass being lost. The fractionation factors used in our model are shown in SI Text section 5.1.

The isotopic composition of water and CO$_2$ lost to transport toward the surface depends on whether diffusion limits the rate of this transport. If diffusion is not rate-limiting, $^{18}R_{\text{evap}} = ^{18}R_{i-1}^{H_2O} \times ^{18}R_{\text{carb}}$—the isotopic ratio of water vapor in equilibrium with the liquid. Similarly, $^{18}R_{\text{gas}} = ^{18}R_{i}^{DIC} \times ^{18}R_{\text{carb}}$—DIC. Diffusive transport imparts an additional fractionation $\delta_{dgas}$, which is multiplied by the values of $R$ shown above and depends on the relative masses of the CO$_2$ and H$_2$O isotopologues.

After modification of the isotopic ratios, the system is isotopically equilibrated at every simulation step. For carbon

$$^{13}R_{i}^{DIC} = f_i^{CO_2(aq)} \times ^{13}R_{i}^{HCO_3(aq)} + f_i^{HCO_3} \times ^{13}R_{i}^{HCO_3} + f_i^{CO_2} \times ^{13}R_{i}^{CO_2},$$  \[S19\]

where

$$f_i^{CO_2(aq)} = \frac{1}{(1 + K_1 + \frac{K_6 x R_{i-1}^{H_2O}}{H_2O_{i-1}})},$$  \[S20\]

$$f_i^{HCO_3} = \frac{K_1}{(1 + K_1 + \frac{K_4 x R_{i-1}^{H_2O}}{H_2O_{i-1}})},$$  \[S21\]

$$f_i^{HCO_3} = \frac{K_6}{(1 + K_1 + \frac{K_4 x R_{i-1}^{H_2O}}{H_2O_{i-1}})}.$$  \[S22\]
The oxygen isotopic ratio of the full system is

\[ R^\text{TOT}_{\text{H}_2\text{O}} = \frac{\mathcal{H}\text{O}_\text{aq}}{\mathcal{H}\text{O}_\text{tot}} = \left[ 55.556 + 2 \times f_i^{\text{CO}_2(aq)} + 3 \times f_i^{\text{H}_2\text{CO}_3} + 3 \times f_i^{\text{CO}_3^-} \right]. \]  

[S27]

The oxygen isotopic ratio of the full system is

\[ 18R^\text{TOT}_{\text{H}_2\text{O}} = \frac{\mathcal{H}_2\text{O}_\text{aq}}{\mathcal{H}_2\text{O}_\text{tot}} = \left[ 55.556 + 2 \times f_i^{\text{CO}_2(aq)} + 3 \times f_i^{\text{H}_2\text{CO}_3} + 3 \times f_i^{\text{CO}_3^-} + f_i^{\text{H}_2\text{CO}_3} \times 18R^\text{TOT}_{\text{H}_2\text{O}} \right]. \]  

[S32]

and the oxygen isotopic ratios of the carbonate species are in equilibrium with that of the water are

\[ 18R^\text{TOT}_{\text{H}_2\text{O}} = 18R^\text{H}_2\text{O} \times 18\sigma_{\text{CO}_2(aq)-\text{H}_2\text{O}}. \]  

[S33]

\[ 18R^\text{H}_2\text{CO}_3 = 18R^\text{H}_2\text{O} \times 18\sigma_{\text{H}_2\text{CO}_3-\text{H}_2\text{O}}. \]  

[S34]

\[ 18R^\text{H}_2\text{O} = 18R^\text{H}_2\text{O} \times 18\sigma_{\text{H}_2\text{O}}. \]  

[S35]

\[ 18R^\text{CO}_3^- = 18R^\text{H}_2\text{O} \times 18\sigma_{\text{CO}_3^--\text{H}_2\text{O}}. \]  

[S36]

Eqs. S2 through S36 can be solved for the oxygen isotopic ratios of all of the aqueous components.

In all of the above calculations, we neglect the effect of salinity on the activities of the different dissolved species and on the isotopic fractionations. We also neglect the effect of aqueous complexes on the activities of the dissolved species. Under the conditions that we explored, the absence of these effects introduces little error, as theionic strength of the solution does not exceed 0.05 in most of the simulations, corresponding to a minimal water activity coefficient of approximately 0.998. The reason for this is that the concentration of the precipitating cations and the carbonate species are limited by the solubility of carbonate minerals. A negligible concentration of non-carbonate-forming ions in solution (e.g., SO\text{2}^-, Cl\text{1}^-, K\text{1}^+, Na\text{1}^+) is justified by the observation that additional (noncarbonate) salts are absent from ALH84001 (8, 9).

In the model, we used the carbonate mineral siderite (FeCO\text{3}) to limit the solubility of precipitating cations and DIC. The fractionations between siderite and water and between siderite and DIC were used to evolve the isotopic composition of the system. This choice is supported by X-ray diffraction and infrared spectra of the carbonates in ALH84001 (6), but below we also show that the results are fairly insensitive to the specific carbonate mineralogy.

### 5.1 References for model constants

We use the following equilibrium constants and isotopic fractionation factors (equilibrium fractionation factors except where noted otherwise).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Constant</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ref. 10)</td>
<td>( K_{\alpha} )</td>
<td>Water dissociation constant</td>
</tr>
<tr>
<td>(ref. 10)</td>
<td>( K_H )</td>
<td>CO\text{2} Henry's Law constant</td>
</tr>
<tr>
<td>(ref. 10)</td>
<td>( K_1 )</td>
<td>True carbonic acid acidity constant</td>
</tr>
<tr>
<td>(ref. 11)</td>
<td>( K_{\alpha} )</td>
<td>First carbonic acid dissociation constant</td>
</tr>
<tr>
<td>(ref. 12)</td>
<td>( K_{\alpha 1} )</td>
<td>Second carbonic acid dissociation constant</td>
</tr>
<tr>
<td>(ref. 13)</td>
<td>( K_{\alpha 2} )</td>
<td>Siderite solubility product constant</td>
</tr>
<tr>
<td>(ref. 14)</td>
<td>( K_{\alpha \text{ siderite}} )</td>
<td>Siderite-water O isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 14)</td>
<td>( K_{\alpha \text{ siderite}} )</td>
<td>Siderite-water C isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 15)</td>
<td>( \sigma_{\text{CO}_2(aq)-\text{H}_2\text{O}} )</td>
<td>CO\text{2(aq)}-water O isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 15)</td>
<td>( \sigma_{\text{CO}_2(aq)-\text{H}_2\text{O}} )</td>
<td>CO\text{2(aq)}-CO\text{2(g)} C isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 16)</td>
<td>( \sigma_{\text{H}_2\text{CO}_3-\text{H}_2\text{O}} )</td>
<td>HCO\text{3(aq)}-water O isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 16)</td>
<td>( \sigma_{\text{H}_2\text{CO}_3-\text{H}_2\text{O}} )</td>
<td>HCO\text{3(aq)}-CO\text{2(g)} C isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 16)</td>
<td>( \sigma_{\text{H}_2\text{O}} )</td>
<td>CO\text{2(g)}-water O isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 16)</td>
<td>( \sigma_{\text{H}_2\text{O}} )</td>
<td>CO\text{2(g)}-CO\text{2(g)} C isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 17)</td>
<td>( \sigma_{\text{H}_2\text{O}} )</td>
<td>CO\text{2(g)}-water O isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 17)</td>
<td>( \sigma_{\text{H}_2\text{O}} )</td>
<td>CO\text{2(g)}-CO\text{2(g)} C isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 18)</td>
<td>( \sigma_{\text{H}_2\text{O}} )</td>
<td>Ice-water O isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 19)</td>
<td>( \sigma_{\text{H}_2\text{O}} )</td>
<td>Kinetic ice-water O isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 20)</td>
<td>( \sigma_{\text{H}_2\text{O}} )</td>
<td>Enstatite-water O isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 20)</td>
<td>( \sigma_{\text{H}_2\text{O}} )</td>
<td>Siderite-water acid digestion O isotope fractionation factor</td>
</tr>
</tbody>
</table>
5.2 Sensitivity analysis. The model parameters are temperature, $f_{\text{degas}}$, initial $p_{\text{CO}_2}$, $A$, and $V$. Temperature is constrained by our clumped isotope measurements to be approximately 18°C. Fig. S6 shows the sensitivity of the system to the other parameters. The sensitivity analysis was done for the default parameter values of $T = 20 \pm 5\,^\circ\text{C}$, $f_{\text{degas}} = 0.9$, $p_{\text{CO}_2} = 0.6$ bar, $A = 0$ mol liter$^{-1}$, and $V = 90\%$ (of the total pore volume). In each case one of the model parameters was varied over a wide range of values and the others left at their default values. The model is highly sensitive only to the value of $f_{\text{degas}}$—a prescribed fraction of the ratio of degassing to precipitation that would be calculated from carbonate chemistry. Physically, this parameter captures the competing rates of carbonate mineral precipitation and carbon loss from the subsurface regolith. A ratio smaller than unity reflects an environment where carbonate precipitation is rapid enough such that loss of carbon from the regolith pores to the surface cannot keep up. Because the combination of carbonate saturation and precipitation requires that carbon is lost from aqueous solution, the CO$_2$ that does not leave the system goes to increase the pressure in the pore space that is unoccupied by the aqueous solution.

We also tested the sensitivity of the modeled $\delta^{18}\text{O}_{\text{H}_{2}\text{O}} - \delta^{13}\text{C}$ covariation to the carbonate mineralogy. The mineralogy affects the chemistry of the aqueous solution (the solubility product constant affects the concentration precipitating cations, the pH, DIC speciation, and gas-aqueous partitioning) as well as the isotopic fractionation associated with carbonate mineral precipitation. Fig. S6D reveals that the choice of carbonate mineralogy (e.g., FeCO$_3$ versus CaCO$_3$) has only a minor impact on the isotopic composition of the precipitates.

5.3 Exploration of alternative scenarios. As mentioned in the model description (Isotopic Evolution Model), the $\delta^{18}$O of the carbonates is buffered by oxygen isotope exchange between the DIC and the water and is, therefore, affected by the processes that fractionate oxygen isotopes in the water. Of these processes we find that only evaporation or vapor-phase diffusion can explain the observed increase in carbonate $\delta^{18}$O over the course of concretion growth (Fig. 4 of the main text). Observed values of $\alpha_{\text{DIC-water}}$ vary between the equilibrium value of 1.00291 and a kinetic fractionation factor of 1.00480 (18). Freezing, therefore, depletes the remaining water in $^{18}$O, rather than enriching it and is also inconsistent with the measured temperatures of carbonate growth. Likewise, at the observed temperatures (including error) and considering the uncertainty in the measured oxygen isotopic composition of the orthopyroxene in ALH84001 ($\delta^{18}$O$_{\text{SMOW}}$ of 4.9 $\pm$ 1.5‰; [6]), oxygen isotope equilibrium between the water and the coexisting silicates can explain carbonate $\delta^{18}$O$_{\text{SMOW}}$ values only as high as approximately 19‰, inconsistent with the full observed range. Furthermore, equilibrium with the silicates would erase the atmospherically derived $\Delta^{17}$O in ALH84001 carbonates.

In addition to the existence of mass-anomalous oxygen and sulfur isotope signatures in ALH84001, we argue that the initial $\delta^{18}$O and $\delta^{13}$C are inconsistent with a deep (magmatic or metamorphic) source for the fluids. If the water came from deeper within the crust, it is expected that the higher temperatures and longer exposure to silicates in the Martian crust would lead to an oxygen isotopic equilibrium between the water and the rock. This is inconsistent with the carbonates’ initial $\delta^{18}$O—at the measured temperature, carbonates precipitated from water in equilibrium with the silicates would have an initial $\delta^{18}$O that is more than 10‰ higher than the lowest value observed in the concretion cores ($\delta^{18}$O$_{\text{SMOW}}$ $\sim$ 5‰; Fig. S7). As for the CO$_2$, based on the $\delta^{13}$C of carbonates in the meteorite Zagami, the $\delta^{13}$C$_{\text{DB}}$ of the magmatic Martian CO$_2$ has been argued to be lower than $-20\%$ (21). At the measured temperature, carbonates in equilibrium with this CO$_2$ would have $\delta^{13}$C$_{\text{PDB}}$ $\sim$ $-9\%$, more than 30‰ lower than the lowest observed values in the concretion cores (approximately 20–25‰). We note, however, that this estimate of Martian magmatic $\delta^{13}$C is not well constrained.

Reliability of Temperature Determination.

1. Resetting of Clumped Isotopic Composition During Recrystallization. The clumped isotopic composition (and the associated temperature) need not always reflect the conditions of original carbonate formation. During diagenetic recrystallization or later exposure to aqueous conditions the carbonates can exchange oxygen isotopes with the water and reach a clumping equilibrium that reflects the temperature of diagenesis or alteration. Electron backscatter diffraction (EBSD) crystal orientation maps of the carbonate concretions reveal a pattern expected from the radial growth of nodules from pore fluids, indicating that the carbonates in ALH84001 have not recrystallized since their ingrowth (Fig. S4). Exchange without recrystallization is unlikely based on the coupled carbon and oxygen isotopic gradient between the concretion cores and rims. Because carbon and oxygen isotopic compositions of carbonate are modified by diagenetic processes differently, it is unlikely that two such processes conspired to produce the observed covariation of $\delta^{18}$O and $\delta^{13}$C across the very large range of values seen in ALH84001 carbonates.

2. Mixing Between Two End Members. Mixing in $\Delta_T$ space is non-linear (22). If two end members have $\delta^{18}$O and $\delta^{13}$C that are sufficiently different (as do the cores and rims of the ALH84001 carbonates), their mixing can yield $\Delta_{T\text{g}}$ values that are much higher than the end member $\Delta_T$ and apparent temperatures that are much lower. For our reliable $\Delta_{T\text{g}}$ measurements (Microvolume Measurements, Standardization, and Error Estimates), if the end members formed at the same temperature but differed in bulk isotopic composition, as the ALH84001 carbonates do, then a best fit of a mixing model to the data yields a temperature of approximately 137.6°C (Fig. S5C). We consider three possible end-member mixing processes that can conspire to give a low apparent temperature of approximately 20°C.

First, the true distribution of carbonate could be bimodal in chemical and isotopic composition, with end members having Mg contents, $\delta^{18}$O and $\delta^{13}$C similar to the very centers of the cores and to the outermost concretion rims (Fig. S5A). If the distribution of chemical and isotopic compositions sampled by bulk acid digestion is different from the true distribution, it could yield spurious $\Delta_{T\text{g}}$ temperature estimates. However, in situ, microscale measurements of chemical and isotopic composition of the carbonates do not reveal two physical end members.

The existence of two chemical end-member aqueous solutions can be rejected on the basis of the time scales for oxygen isotopic equilibration in aqueous solution as a function of pH and temperature. Essentially, the carbonates would have to precipitate after the mixing the two end members but before oxygen isotopic equilibrium (and consequently $\Delta_{T\text{g}}$ equilibrium) was reached. The time to 99% equilibration as a function of pH and temperature is shown in Fig. S5D. The results of this calculation at temperatures exceeding 50°C are less reliable because the equilibrium constants in the carbonate system have more uncertainty at these temperatures. They do, however, give a sense of the time scales required for almost full isotopic equilibrium. If the temperature was indeed approximately 137.6°C, the carbonates would have to precipitate in a matter of seconds. We find this scenario unlikely.

A third possibility is that the acid digestion preferentially sampled the tails of a distribution of chemical and isotopic composition like that observed in ALH84001 (Fig. S5B). Physical mixing of the two gases evolved in this way in the absence of an aqueous solution in which they could approach $\Delta_{T\text{g}}$ equilibrium would yield a spuriously low apparent temperature. However, we digested the carbonate for a total of 12 h at 90°C—long


Mittlefehldt DW (1994) ALH84001, a cumulate orthopyroxenite member of the Martian meteorite clan. Meteoritics 29:214–221.


Fig. S1. Experimental apparatus. Crushed bulk ALH84001 was loaded into glass boats and placed in the glass side arm protruding (Left). A glass-coated magnet was placed behind (to the left of) the sample boats. Phosphoric acid (102%) was placed in the bottle, along with a Teflon-coated magnetic stir bar. Apiezon H vacuum grease was used on all of the ground glass joints to seal the reaction vessel and the apparatus was pumped on for more than 16 h (at a heating block temperature of 105 °C) to get rid of water. Before the digestion, the heating block temperature was lowered to 90 °C and given enough time to reach this temperature. The glass-coated magnet was used to push the sample boats into the acid and start the digestion. The CO$_2$ was continuously collected in liquid nitrogen after passing through a coiled glass trap held at −75 °C (ethanol-dry ice slush).
Fig. S2. Microvolume schematic and comparison of results with dual-inlet measurements of the same gas. (A) The microvolume. The CO$_2$ is frozen from the bellows into the small electroformed nickel U trap by immersion in liquid N$_2$. Valve B is then closed, the liquid N$_2$ removed, and the microvolume bathed in hot air in order for the CO$_2$ to thaw (this takes less than 30 s). The CO$_2$ flows down the pressure gradient in the capillary tube into the ion source. (B) Raw $\Delta$47 (uncorrected for mass spectrometer artifacts and compositional dependence on $\delta$47) plotted against measurement voltage (on the faraday cup measuring mass 44 CO$_2$). The corrected $\Delta$47 is always positive and slightly elevated relative to the true known value, for reasons discussed in SI Text. (C) $\delta$13C plotted against voltage. (D) $\delta$18O plotted against measurement voltage. In all three plots, unfilled and filled circles are the individual dual-inlet measurements and their average, respectively. Unfilled squares are the microvolume measurements and filled squares are their voltage-weighted average, plotted at a voltage of 7 V for ease of comparison with the dual-inlet measurements. The $\delta$18O and $\delta$13C are well-captured by the voltage-weighted average, whereas the $\Delta$47 is systematically lower and closer to the true known value of $\Delta$47 for these carbonate standards.

Fig. S3. $\Delta$47 versus $\delta$47 for gases of variable bulk isotopic composition heated to 1,000 °C to achieve a random distribution of the heavy isotopes. These gases should all have $\Delta$47 = 0‰, and it is not entirely clear why they do not. However, it has been established that a sample’s true value of $\Delta$47 is retrieved if the measured value is corrected by the $\delta$47-dependent amount represented by a fit to these $\delta$47-$\Delta$47 data. Here we show that over the range of $\delta$47 observed in ALH84001 carbonates (approximately 35–50‰; gray shaded areas), the relationship between $\delta$47 and $\Delta$47 of heated gases (and therefore the correction) is linear. The three subfigures show the “heated gas lines” for three different measurement periods (December 23–31, 2005; November 18, 2005; and February 18–24, 2006). We thank Weifu Guo, who performed these analyses, for the data.
Fig. S4. Electron backscatter diffraction image of a carbonate concretion from the same split of ALH84001 from which we extracted carbonates for clumped isotope analysis. (Inset) The orientation of the carbonate c axis sweeping in a fan-like manner, consistent with radial growth of the concretion. Recrystallization would appear as a juxtaposition of crystal domains with an orientation of the carbonate c axis that is uncorrelated between adjacent domains. The absence of such a pattern indicates that the carbonates in ALH84001 have not recrystallized since their formation.

Fig. S5. The effect of two end-member mixing on the measured $\Delta_{47}$. Schematic models for (A) physical or chemical mixing and (B) mixing during acid digestion of two end members with different $\delta^{18}O$ and $\delta^{13}C$ (and Mg content) and identical formation temperatures (and $\Delta_{47}$). See Mixing Between Two End Members for details. (C) The $\Delta_{47}$ of a mixture of two end members with $\delta^{18}O_{SMOW}$ of 5 and 30‰, and $\delta^{13}C_{PDB}$ of 20 and 65‰, similar to the bulk isotopic composition of the concretion cores and rims, respectively. Our reliable analyses (Microvolume Measurements, Standardization, and Error Estimates) are best fit by a mixture of these two isotopic end members that formed at a temperature of 137.6 °C. Mixtures of end members formed at other temperatures (dashed lines) are compared to the data (filled circles). (D) The time to 99% oxygen isotope equilibration as a function of temperature and pH. Kinetic rate constants for the relevant reactions are from ref. 10. Equilibration proceeds more rapidly at high temperature, though we note that the equilibrium constants in the carbonate system are best constrained at temperatures below 50 °C with the calculations at higher temperatures more uncertain. The gray rectangle brackets the range of pH reached in our calculations, which is a function of the partial pressure of CO$_2$ and the initial nonprecipitating ion load. At the best fit mixing temperature (approximately 137.6 °C, Fig. S5C), the carbonates would have to precipitate on a time scale of seconds for oxygen isotope (and $\Delta_{47}$) equilibrium not to be reached—that is, to sustain the spuriously low apparent temperatures due to end-member mixing.
Fig. S6. Model sensitivity analysis. In all cases the sensitivity of the modeled $\delta^{13}C$ and $\delta^{18}O$ evolution to the value of a single parameter is shown. We demonstrate the sensitivity of the pore $pCO_2$ increase and the pH decrease only to the value of $f_{dgas}$, but give the maximal increase in pore $pCO_2$ on the lower right of each subfigure. (A) Sensitivity to the value of $f_{dgas}$, the fraction of noncarbonate carbon lost to transport toward the surface (the rest goes to increase the pore $pCO_2$). The model results are sensitive to the value of this parameter because the ratio of degassing to precipitation determines the net fractionation of carbon isotopes. Carbonate mineral precipitation depletes the remaining DIC, whereas $CO_2$ degassing enriches it in $^{13}C$. (B) Sensitivity of the $pCO_2$ increase (thick lines, right y axis) and pH decrease (thin lines, left y axis) to the value of $f_{dgas}$. The line styles and colors correspond to the legend in A. A low value of $f_{dgas}$ results in the largest increase in pore $pCO_2$, but still less than a 15% increase. (C) Sensitivity to the initial charge due to the concentration of nonprecipitating, conservative ions (parameter A). (D) Sensitivity to carbonate mineralogy, represented in the model by the value of $K_{SP}$ and of the fractionation factors between the carbonate mineral and the water and $CO_2$ from which it formed. (E) Sensitivity to initial $pCO_2$. (F) Sensitivity to the initial volume of pore space occupied by the aqueous solution (the rest of the volume is available for gaseous $CO_2$).

Fig. S7. Comparison of the $\delta^{18}O$ of water in equilibrium with the observed range of carbonate $\delta^{18}O$ to that of water in equilibrium with the silicates in ALH84001 at 20 °C. The carbonates start out too $^{18}O$-depleted and end up too enriched in $^{18}O$ for oxygen isotope exchange between the water and rock to explain the core to rim evolution of $\delta^{18}O$. In addition, the low $\delta^{18}O$ of the carbonates (relative to the expectation from equilibrium with the silicates in ALH84001) suggests that the water from which the carbonates precipitated did not come from deeper within the Martian crust, but from the surface environment.
Table S1. Correction to δ¹⁸O, δ¹³C, and Δ⁴⁷ measured from the microvolume

<table>
<thead>
<tr>
<th>Period</th>
<th>Standard*</th>
<th>Weight, mg</th>
<th>Δδ¹⁸O, ‰</th>
<th>Δδ¹³C, ‰</th>
<th>ΔΔ⁴⁷, ‰</th>
<th>N₁₇²V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ext. B</td>
<td>CM</td>
<td>6.9</td>
<td>-0.007</td>
<td>-0.009</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CM</td>
<td>6.9</td>
<td>-0.061</td>
<td>-0.086</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CM</td>
<td>7.1</td>
<td>-0.169</td>
<td>-0.085</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CM</td>
<td>3.9</td>
<td>-0.153</td>
<td>-0.062</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CM</td>
<td>3.9</td>
<td>-0.239</td>
<td>-0.113</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TV</td>
<td>7.1</td>
<td>-0.288</td>
<td>-0.108</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>2.5</td>
<td>—</td>
<td>—</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>4.5</td>
<td>0.181</td>
<td>-0.019</td>
<td>-0.023</td>
<td></td>
</tr>
</tbody>
</table>

*The internal standards used for determination of the correction were Carrera Marble (CM), a travertine (TV), and Inyo Dolomite (ID). The standards cover a range of D47 between 0.352 and 0.662. 95% confidence interval error estimate after application of the correction.

Table S2. All results, including aliquot A

<table>
<thead>
<tr>
<th>Type</th>
<th>δ¹⁸O, ‰ C</th>
<th>δ¹⁸O, ‰ N</th>
<th>δ¹³C, ‰ C</th>
<th>δ¹³C, ‰ N</th>
<th>ΔΔ⁴⁷, ‰ C</th>
<th>ΔΔ⁴⁷, ‰ N</th>
<th>N₁₇²V</th>
</tr>
</thead>
<tbody>
<tr>
<td>μV</td>
<td>13.05</td>
<td>—</td>
<td>35.52</td>
<td>—</td>
<td>0.555</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>μV</td>
<td>16.68</td>
<td>—</td>
<td>40.87</td>
<td>—</td>
<td>0.522</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>μV</td>
<td>18.96</td>
<td>—</td>
<td>40.82</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Aliquot B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>μV</td>
<td>13.29</td>
<td>13.38 ± 0.28</td>
<td>34.54</td>
<td>34.54</td>
<td>34.60 ± 0.07</td>
<td>0.657</td>
<td>0.642</td>
</tr>
<tr>
<td>μV</td>
<td>16.86</td>
<td>—</td>
<td>40.99</td>
<td>—</td>
<td>41.05 ± 0.07</td>
<td>0.653</td>
<td>0.638</td>
</tr>
<tr>
<td>μV</td>
<td>17.90</td>
<td>17.99 ± 0.28</td>
<td>40.87</td>
<td>40.94 ± 0.07</td>
<td>0.757</td>
<td>0.742</td>
<td>1</td>
</tr>
<tr>
<td>Aliquot C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>μV</td>
<td>14.07</td>
<td>—</td>
<td>35.12</td>
<td>—</td>
<td>35.14 ± 0.02</td>
<td>0.740</td>
<td>0.693</td>
</tr>
<tr>
<td>μV</td>
<td>16.23</td>
<td>16.27 ± 0.16</td>
<td>40.67</td>
<td>40.67 ± 0.02</td>
<td>0.690</td>
<td>0.643</td>
<td>9</td>
</tr>
</tbody>
</table>

The columns from left to right are measurement type (Δ = dual-inlet, μV = microvolume), uncorrected δ¹⁸O, corrected δ¹⁸O with error (see Table S1), uncorrected δ¹³C, corrected δ¹³C with error, uncorrected Δ⁴⁷, corrected Δ⁴⁷, number of acquisitions, Δ⁴⁷ error estimated from carbonate standards, Δ⁴⁷ counting statistics error, temperature with error. Details of error estimation are in SI Text section Microvolume Measurements, Standardization, and Error Estimates.