Carbonates in the Martian meteorite Allan Hills 84001 formed at 18 ± 4 °C in a near-surface aqueous environment

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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 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 47 CO2 (mostly 13C18O16O) evolved from acid digestion of carbonate minerals, is higher than predicted from a random distribution of the heavy isotopes. This excess is denoted Δ17 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).

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Table 1. Isotopic composition of ALH84001 carbonates extracted by stepped acid digestion

<table>
<thead>
<tr>
<th>Aliquot</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>δ¹³C‰</td>
<td>Δγ/°C</td>
</tr>
<tr>
<td>Aliquot A</td>
<td>—</td>
<td>13.05</td>
<td>35.52</td>
</tr>
<tr>
<td>(320 mg)</td>
<td></td>
<td></td>
<td></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>

δ¹³O and δ¹³C are reported relative to the SMOW and PDB 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 A 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 δ¹³O and δ¹³C values of the carbonate extracted in the 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 δ¹³O and δ¹³C measurements with prior constraints on the relationship between 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 Δγ measurements, but has δ¹³O and δ¹³C values that are consistent with subsequent extractions of two larger aliquots (1,625 and 1,550 mg, respectively). The Δγ measurements of these two larger aliquots (Table 1) yield six temperatures between 31±25° and 30±13°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 Δγ 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 concretions have not been subject to recrystallization since their radial growth from aqueous solutions. In the absence of recrystallization, resetting of the carbonates’ isotopic composition should yield the observed, strongly covarying relationship between δ¹³O and δ¹³C is unlikely. Together, these observations indicate that the measured Δγ 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 δ¹³C and δ¹³O 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 Δγ data can be rejected on the basis of the relationships observed among the δ¹³O, δ¹³C, and Δγ measured in the carbonate (Fig. 2). Carbonate precipitation over a range of temperatures (e.g., ref. 22) does not fit the observed δ¹³O and δ¹³C array (Fig. 2A). Because the stepped acid digestion could not perfectly separate the concretion cores from their rims, the range of δ¹³O_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 δ¹³O–Δγ data (aliquots B and C) is large at the ends of the full δ¹³O 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 Δγ that are lower than the true values by as much as 0.12‰. Applied to our measured Δγ, this would translate into an error-weighted average temperature of carbonate formation in ALH84001 of ~7°C (instead of approximately 18°C if the Δγ 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 δ¹³O 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 δ¹³O and δ¹³C values and correlations between major element and isotopic composition (see text).
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.

**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 Δ_{47} constraints, we find that the striking core to rim coevolution of δ^{18}O_{SMOW} (approximately 5 → 30‰) and δ^{13}C_{PDB} (approximately 25 → 65‰), observed in previous ion-microprobe studies (15–18) is best explained by gradual evaporation of a shallow subsurface aqueous solution at a constant, low temperature. The evaporative water loss drives carbonate precipitation 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 δ^{18}O and δ^{13}C 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 δ^{18}O and δ^{13}C 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

![Fig. 2.](image)

Comparing δ^{13}C and δ^{18}O with models for the isotopic variation in ALH84001 carbonates. (A) Relationship between δ^{13}C and δ^{18}O. The solid black line and the broken black curves are the 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 δ^{13}C and δ^{18}O observed in microprobe studies (approximately 5–30‰). A hypothesis of evolving temperatures can be rejected on the basis of the misfit between these lines. (B) Relationship between Δ_{47} and δ^{18}O. 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 Δ_{47} and δ^{18}O observed in microprobe studies (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 models predictions for the relationship between Δ_{47} and δ^{18} O 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 δ^{13}C and δ^{18}O) and only marginally include kinetic isotope effects due to rapid degassing (see text).

![Fig. 3.](image)

Model for carbonate formation in ALH84001. (A) Physical model of a shallow subsurface aquifer. The depth of carbonate formation is constrained by a combination of the cosmic ray exposure pattern of ALH84001 and Martian meteorite ejection models (48, 49). (B) Reduction of the physical model to a geochemical model of carbonate precipitation and CO₂ degassing driven by gradual evaporation of water in a confined volume, coupled to loss of the vapor phase.
Hypotheses that explain the observed isotopic trends with evaporative volcanic fields in Arizona (45). All of these studies discuss hydrated deserts (43, 44), and caliche formed on basalts in young space (41), as do endostromatolites in an impact structure in the Atacama deserts (43, 44). These analogs are imperfect because even in cold or arid regions, the fluid in an environment such as the one we describe could, in principle, come from depth (magmatic or hydrothermal fluid), there is evidence that the parent fluid of the ALH84001 carbonates came from the surface (atmosphere–hydrosphere). The carbonates have an oxygen isotopic composition that is anomalous with respect to Martian igneous rocks ($\Delta^{18}O > 0.3 \pm 0.0$ (46)). Similarly, sulfides in this sample have a “mass-independent” sulfur isotopic composition ($\Delta^{34}S \neq 0$ (47)).

Support for the hypothesis of carbonate formation from an evaporating subsurface aqueous reservoir comes from observed covariation between $\delta^{13}C$ and $\delta^{18}O$, but is not supported by the observed $\delta^{13}C - \Delta^{17}O$ relationship (Fig. 2B). Finally, although the observed $\delta^{13}C$ increase can also be explained by organic matter synthesis or oxidation, the existence of extraterrestrial organic matter in ALH84001 remains controversial, due mainly to the difficulty in ruling out contamination by terrestrial organic matter (27). Furthermore, the covariation in carbon and oxygen isotope ratios suggests a common process affected both reservoirs.

Support for the hypothesis of carbonate formation from an evaporating subsurface aqueous reservoir comes from observed covariation between $\delta^{13}C$ and $\delta^{18}O$ in terrestrial carbonates from cold or arid regions. These analogs are imperfect because even in cold, dry, and relatively organic-poor environments, organic matter fixation or respiration contributes to the preserved isotopic signal to variable degree. Even so, the isotopic composition of Mg-bearing carbonates formed on ultramafic mine tailings in northwestern Canada (latitude $\pm 58^\circ$N) form arrays strikingly similar to those observed in ALH84001 carbonates in $\delta^{13}O$-$\delta^{13}C$ space (41), as do endostromatolites in an impact structure in the Canadian high Arctic (42), soil carbonates from the Mojave and Atacama deserts (43, 44), and caliche formed on basalts in young volcanic fields in Arizona (45). All of these studies discuss hypotheses that explain the observed isotopic trends with evaporation and CO$_2$ degassing.

To further examine this hypothesis we developed a model of carbonate precipitation (Fig. 3B) in which carbonate mineral saturation and charge balance are maintained, as is chemical and isotopic equilibrium among the aqueous solution, the instantaneous precipitate, and the gas phase in the regolith during gradual evaporation (Methods and SI Text). The range of $\delta^{13}O$ values in the concretions requires distillative loss of 42–92% of the water, depending on whether the transport of water toward the surface is rate limited by vapor-phase diffusion. The observed covariation in $\delta^{18}O$ and $\delta^{13}C$ can be reproduced (Fig. 4) if approximately 87–98% of the noncarbonate carbon loss from solution is transported to the surface. The remaining 2–13% causes a pore CO$_2$ increase of 5–15%, consistent with the modest pressure gradient that would be required to cause distillative CO$_2$ loss from the subsurface in the scenario considered here. These results are relatively insensitive to the choice of initial pCO$_2$, the nonprecipitating ion concentration, and the particular carbonate mineralogy (SI Text and Fig. S6).

Although the fluid in an environment such as the one we describe could, in principle, come from depth (magmatic or hydrothermal fluid), there is evidence that the parent fluid of the ALH84001 carbonates came from the surface (atmosphere–hydrosphere). The carbonates have an oxygen isotopic composition that is anomalous with respect to Martian igneous rocks ($\Delta^{18}O > 0.3 \pm 0.0$ (46)). Similarly, sulfides in this sample have a “mass-independent” sulfur isotopic composition ($\Delta^{34}S \neq 0$ (47)). Both signatures were likely generated by atmospheric reactions and support a surface source of the fluid. Furthermore, water in oxygen isotope equilibrium with the Ca-rich concretion cores (determined by microprobe studies) would, at the temperatures we measured, have $\delta^{18}O$ more than 10‰ lower than equilibrium with coexisting silicate minerals (Fig. S7) and CO$_2$ in equilibrium with the concretion cores is tens of permil too high in $\delta^{13}C$ to have come directly from the Martian mantle (though the $\delta^{13}C$ of the Martian mantle is poorly constrained, SI Text).

At the measured growth temperature, the Mg-rich carbonates in the concretion rims have $\delta^{13}O$ 10–15‰ heavier than equilibrium with the silicates (Fig. S7). This, together with the persistence of the mass-anomalous oxygen isotope composition, implies that the combination of time and temperature was insufficient to allow appreciable oxygen isotope exchange between water and rock, supporting a low-temperature, transient aqueous environment. This is consistent also with the absence of alteration minerals (e.g., hydrous phyllisilicates) in ALH84001 other than the carbonates and the associated sulfides and magnetite (13, 14).

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 fastest-reacting, Ca-rich carbonates, whereas the second and third steps (1–4 and 4–12 h, respectively) sampled more Mg-rich compositions (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 contaminations.
tion, and the precious nature of the sample. The bulk and clumped isotopic composition of evolved CO2 was measured on a Thermo Finnigan MAT 253 isotope ratio gas source mass spectrometer. The small sample size necessitated measurement using a microvolume. Measurements were standardized to carbonates of known bulk and clumped isotopic composition, treated identically to the meteorite aliquots and measured using a microvolume. See SI Text for full analytical methods.

With knowledge of the precipitation temperature, we constructed an isotope evolution model and examined hypotheses for carbonate precipitation that could also quantitatively explain the observed covariation of carbon and oxygen isotope ratios in the carbonate. Prescribing values for the model-free parameters—the initial CO2 pressure (pCO2) and the concentration of non-precipitating ions—we solve for the pH, solute concentrations, and pCO2 as the water evaporates. The oxygen isotopic evolution depends on the cumulative fraction of water lost to evaporation and vapor-phase diffusion.

The 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.

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Supporting Information

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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 valved glass sample vial immersed in liquid N₂. The sample vials were immediately taken to analysis. The analyte CO₂ was carried on a helium flow through 0.3 m of ⅛″ 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-O™ (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 acquisition to match the gas pressure 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 δ¹³O, δ¹⁸O, and ΔTD relative to low-pressure dual-inlet measurements of the same samples (Fig. S2B−D). The δ¹³O became progressively heavier in all experiments by as much as 2‰, whereas the δ¹⁸O 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 δ¹³O 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 δ¹³O and δ¹⁸O of the standard materials was retrieved by calculating an average of the δ¹³O and δ¹⁸O measured out of the microvolume, weighted by the average voltage (on the faraday cup measuring mass 44 CO₂) out of each acquisition (Fig. S2B−D). A small offset remained from the values measured in dual-inlet mode for both δ¹³O and δ¹³C (Table S1). After correction for this offset, the 95% confidence interval on the remaining error was ±0.281‰ and ±0.072‰ for δ¹³O 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 δ¹³O 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 ΔTD 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 ΔTD. The correction is a linear function of ΔTD, even up to the very high values of ΔTD observed in ALH84001 (approximately 50‰; Fig. S3). After this correction, the value of ΔTD of small samples was found to be positively offset from the true known value. It is not known how clumped isotope abundances behave in CO₂ ice/vapor systems, but this increase in ΔTD of small samples occurred 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 ΔTD 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 ΔTD 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 ΔTD measured for the ALH84001 carbonates. The absolute value of the remaining error on the ΔTD 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 ΔTD 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 ΔTD during the period of extraction A, as the standardization had not yet been applied.

A second error estimate for ΔTD 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 isotopic compositions, with δ18O of approximately 0.8%e (3). To account for this, we used the raw ratios of mass 45 and 46 CO2 to mass 44 CO2 (45R and 46R, respectively) to solve the following three equations for the samples’ isotopic ratios 13R, 17R, and 18R (13C/12C, 17O/16O, and 18O/16O, respectively):

\[
\begin{align*}
45R &= 13R + 2\Delta_{17}R, \\
46R &= 2 \times 18R + 2 \times 17R + 17R^2, \\
17R &= K_1 \times 18R^2 + K_2,
\end{align*}
\]

where \(K_1 = 17_{RVSMOW}/(18_{RVSMOW})^2\), \(K_2 = 17_{RVSMOW} \times \Delta_{17O}/1,000\), \(\Delta_{17}\) is the slope of the oxygen isotope mass-fractionation line, and \(18_{RVSMOW}\) is the ratio \(18O/16O\) in VSMOW (Vienna Standard Mean Ocean Water). These values of 13R, 17R, and 18R were then used to calculate the random distribution of the heavy isotopes, which was used in the calculation of \(\Delta_{17}\). With a \(\Delta_{17O}\) of 0.8%e, the \(\Delta_{17}\) increased by approximately 0.035%e, corresponding to a temperature decrease of approximately 7°C (relative to the same calculation carried out with \(\Delta_{17O}\) of 0%e). The sample’s δ18O and δ17O typically increased by approximately 0.027% and approximately 0.24%e, respectively.

4. Major Element Composition Estimates. The microprobe δ18O and δ17C data (4–7) were correlated by Mg content in six bins of Mg normalized mole fraction (Xmg), each with a width of 0.1. The average and two standard deviations of the δ18O and δ17C in each of the six bins are shown in Fig. 4 in the main text (gray squares). A weighted least squares linear fit with 95% confidence bounds is also shown in Fig. 4. We used this fit, together with the δ17C—major element composition data to generate a synthetic dataset consisting of 10⁶ points, as follows: Combinations of \(\delta^{18}O\times X_{mg}\times X_{Fe}\times X_{Ca}\) 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 δ17C 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 \(X_{mg}\), \(X_{Fe}\), and \(X_{Ca}\) given the δ18O and δ17C measured in our bulk carbonate acid digestions (Fig. 1 in the main text).

5. Isotopic Evolution Model. The observed carbon, oxygen, and Δ17 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 δ17C 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 H2O to CO2 exceeding 10³. For example, even if the ancient Martian atmosphere contained 1 bar of CO2, a solution with no dissolved cations (i.e., where the partial pressure of CO2 alone controlled the solution pH) would have a water to CO2 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 CO2. For example, saturation with sidereite (FeCO3) at pCO2 of 1 bar requires 1.7 × 10⁻³ mol per liter of Fe²⁺. This concentration of dissolved ferrous iron would increase the pH only to approximately 5.0 and decrease the water to CO₂ 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 CO₂ ratio to approximately 1,060. Thus, the δ18O of carbonates precipitated from an aqueous solution formed at the surface of early Mars would be water-buffered. Under equilibrium conditions, the carbonates’ δ18O would be controlled by the processes affecting the δ18O 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 CO2 degassing. The distillative loss of water to evaporation and CO2 to degassing cause an increase in the δ18O and δ17C of the residual fluid, respectively. In this system, the total dissolved inorganic carbon, DIC (in moles), can be expressed in two ways:

\[
\text{DIC} = p\text{CO}_2 \times K_H \times \left(1 + \frac{K_{A1}}{\text{H}^+} + \frac{K_{A2}}{\text{H}^+} \right) \times \text{H}_2\text{O}. 
\]

\[
\text{DIC} = \frac{K_{SP}}{\text{M}^{2+} / \text{H}_2\text{O}} \times \left(1 + \frac{\text{H}^+}{K_{A2}} + \frac{\text{H}^+}{K_{A1}} \right) \times \text{H}_2\text{O}. 
\]

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, MCO3, \(K_H\), \(K_{A1}\), \(K_{A2}\), and \(K_{SP}\) are Henry’s Law constant for CO2, the first and second carbonic acid dissociation constants, and the solubility product constant for MCO3, respectively (SI Text section References for model constants). \([\text{H}^+]\) is the concentration of the hydronium ion (in moles per liter), \(\text{M}^{2+}\) is the amount of the precipitating cation (in moles), \(\text{H}_2\text{O}\) is the amount of water (in liters), and pCO2 is the pore CO2 pressure (in atmospheres).

Equating these two expressions for DIC, we obtain

\[
p\text{CO}_2 \times K_H \times K_{A1} \times K_{A2} \times \frac{1}{\text{H}^+} = \frac{K_{SP}}{\text{M}^{2+} / \text{H}_2\text{O}}. 
\]

Charge balance dictates

\[
\text{H}^+ - [\text{OH}^-] - 2 \times [\text{CO}_3^{2-}] + 2 \times \frac{\text{M}^{2+}}{\text{H}_2\text{O}} + A = 0, 
\]

and, given carbonate species equilibrium, can be written as

\[
\text{H}^+ = \frac{K_H}{[\text{H}^+]} - \frac{p\text{CO}_2 \times K_H \times K_{A1} \times K_{A2}}{[\text{H}^+]} - 2 \times \frac{p\text{CO}_2 \times K_H \times K_{A1} \times K_{A2}}{[\text{H}^+]^2} + 2 \times \frac{\text{M}^{2+}}{\text{H}_2\text{O}} + A = 0. 
\]
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 \( p_{CO_2}, A, \) 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 \( p_{CO_2}, A, \) 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,

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

\[
\Delta M^{2+} = M_{i-1}^{2+} - M_i^{2+}, \quad [S10]
\]

where \( M_{i-1}^{2+} \) and \( M_i^{2+} \) are the amount of precipitating cation solved for with \( H_2O_{i-1} \) and \( H_2O_i \) liters of water, respectively. The total DIC loss is then

\[
\Delta DIC = DIC_{i-1} - DIC_i = \Delta DIC_{carb} + \Delta DIC_{dgas} + \Delta DIC_{pres}. \quad [S11]
\]

where \( DIC_{i-1} \) and \( DIC_i \) are the total DIC solved for with \( H_2O_{i-1} \) and \( H_2O_i \) 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 \( p_{CO_2} \). 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,

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

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

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

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 \( p_{CO_2} \) then evolves as

\[
p_{CO_2} = p_{CO_2_{i-1}} + \frac{\Delta DIC_{pres} \times N_A \times k_B \times T}{V}, \quad [S15]
\]

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 \( p_{CO_2} \) 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 \( \Delta \) 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 reequilibration isotopic composition of the water and DIC reservoirs is

\[
18R_i^{H_2O} \times H_2O = 18R_i^{\Delta H_2O} \times H_2O_{i-1} - \Delta H_2O \times \delta^{18}Revap, \quad [S16]
\]

\[
18R_j^{DIC} \times DIC_j = 18R_j^{\Delta DIC} \times DIC_{i-1} - \Delta DIC_{i-1} - \Delta DIC_{carb} \times 18R_j^{carbonate} - \Delta DIC_{gas} \times 18R_j^{gas} - \Delta DIC_{pres} \times \delta^{18}Revap. \quad [S17]
\]

\[
13R_j^{DIC} \times DIC_j = 13R_j^{\Delta DIC} \times DIC_{i-1} - \Delta DIC_{i-1} - \Delta DIC_{carb} \times 13R_j^{carbonate} - \Delta DIC_{gas} \times 13R_j^{gas} - \Delta DIC_{pres} \times \delta^{13}Revap, \quad [S18]
\]

where \( \delta^{18}Revap \) is the ratio of \( ^{18}O \) to \( ^{16}O \) in reservoir \( x \) at step \( i \) and \( \delta^{13}Revap \) 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, \( \delta^{18}Revap = \delta^{18}H_2^{gas} \times \delta^{18}Revap_{\alpha-\lambda} \) the isotopic ratio of water vapor in equilibrium with the liquid. Similarly, \( 18R_j^{\Delta DIC} = 18R_j^{DIC} \times 18R_j^{\alpha-\Delta DIC} + 18R_j^{\alpha-\Delta DIC} \) Diffusive transport imparts an additional fractionation \( \alpha_{\Delta DIC} \), which is multiplied by the values of \( \alpha \) shown above and depends on the relative masses of the \( CO_2 \) and \( H_2O \) isotopologues.

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

\[
13R_j^{DIC} = f_i^{CO_2(\alpha)} \times 13R_j^{CO_2(\alpha)} + f_i^{HCO_3^-} \times 13R_j^{HCO_3^-} + f_i^{HCO_3^-} \times 13R_j^{HCO_3^-} + f_i^{CO_3^-} \times 13R_j^{CO_3^-}, \quad [S19]
\]

where

\[
f_i^{CO_2(\alpha)} = \frac{1}{(1 + K_1 + \frac{K_2}{[H^+]})}, \quad [S20]
\]

\[
f_i^{HCO_3^-} = \frac{K_1}{(1 + K_1 + \frac{K_2}{[H^+]})}, \quad [S21]
\]

\[
f_i^{HCO_3^-} = \frac{K_2}{(1 + K_1 + \frac{K_2}{[H^+]})}. \quad [S22]
\]
\[ f_i^{\text{CO}_2^=} = \frac{K_{i,\text{aq}} \times K_{i}}{(1 + K_{i} \times \frac{K_{i,\text{aq}}}{[\text{H}^+]})} \]

The carbonate species are in isotopic equilibrium:

\[ 13^R_i \text{CO}_2^{(aq)} = 13^R_i \text{HCO}_3^- \times 13^R \Delta_{\text{CO}_2^{(aq)}-\text{HCO}_3^-} \quad \text{(Eqs. S24)} \]
\[ 13^R_i \text{HCO}_3^- = 13^R_i \text{CO}_3^{2-} \times 13^R \Delta_{\text{HCO}_3^--\text{CO}_3^{2-}} \quad \text{(Eqs. S25)} \]
\[ 13^R_i \text{CO}_3^{2-} = 13^R_i \text{HCO}_3^- \times 13^R \Delta_{\text{CO}_3^{2-}-\text{HCO}_3^-} \quad \text{(Eqs. S26)} \]

Eqs. S19 and S24 through S26 can be solved for the carbon isotopic composition of all of the carbonate species. For oxygen, water is included in the equilibrium. The total number of moles of oxygen in the solution is

\[ \text{H}_2\text{O}\{55.5556 + 2 \times [\text{CO}_2^{(aq)}] + 3 \times [\text{H}_2\text{CO}_3] + 3 \times [\text{HCO}_3^-] + 3 \times [\text{CO}_3^{2-}] \}. \quad \text{(S27)} \]

\[ [\text{CO}_2^{(aq)}] = f_i^{\text{CO}_2^{(aq)}} \times \text{DIC}_{\text{H}_2\text{O}}^- \quad \text{(S28)} \]
\[ [\text{H}_2\text{CO}_3] = f_i^{\text{H}_2\text{CO}_3} \times \text{DIC}_{\text{H}_2\text{O}}^- \quad \text{(S29)} \]
\[ [\text{HCO}_3^-] = f_i^{\text{HCO}_3^-} \times \text{DIC}_{\text{H}_2\text{O}}^- \quad \text{(S30)} \]
\[ [\text{CO}_3^{2-}] = f_i^{\text{CO}_3^{2-}} \times \text{DIC}_{\text{H}_2\text{O}}^- \quad \text{(S31)} \]

The oxygen isotopic ratio of the full system is

\[ ^{18}\text{R}_i^{\text{TOT}} = \frac{\text{H}_2\text{O}}{^{18}\text{R}_i^{\text{TOT}}} \times \{55.5556 + 2 \times f_i^{\text{CO}_2^{(aq)}} \times ^{18}\text{R}_i^{\text{CO}_2^{(aq)}} + \ldots + 3 \times (f_i^{\text{H}_2\text{CO}_3} \times ^{18}\text{R}_i^{\text{H}_2\text{CO}_3} + f_i^{\text{HCO}_3^-} \times ^{18}\text{R}_i^{\text{HCO}_3^-} + f_i^{\text{CO}_3^{2-}} \times ^{18}\text{R}_i^{\text{CO}_3^{2-}}) \}. \quad \text{(S32)} \]

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

\[ ^{18}\text{R}_i^{\text{CO}_2^{(aq)}} = ^{18}\text{R}_i^{\text{H}_2\text{O}} \times ^{18}\text{R}_{\text{CO}_2^{(aq)}-\text{H}_2\text{O}} \quad \text{(S33)} \]
\[ ^{18}\text{R}_i^{\text{HCO}_3^-} = ^{18}\text{R}_i^{\text{H}_2\text{O}} \times ^{18}\text{R}_{\text{HCO}_3^--\text{H}_2\text{O}} \quad \text{(S34)} \]
\[ ^{18}\text{R}_i^{\text{CO}_3^{2-}} = ^{18}\text{R}_i^{\text{H}_2\text{O}} \times ^{18}\text{R}_{\text{CO}_3^{2-}-\text{H}_2\text{O}} \quad \text{(S35)} \]
\[ ^{18}\text{R}_i^{\text{CO}^-} = ^{18}\text{R}_i^{\text{H}_2\text{O}} \times ^{18}\text{R}_{\text{CO}^--\text{H}_2\text{O}} \quad \text{(S36)} \]

Eqs. S32 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 the ionic 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{aq}^2-, Cl\text{aq}^-, K\text{aq}^+, Na\text{aq}^+) 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{aq}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>Description</th>
</tr>
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<tbody>
<tr>
<td>(ref. 10)</td>
<td>Water dissociation constant</td>
</tr>
<tr>
<td>(ref. 10)</td>
<td>CO\text{aq}2- Henry's Law constant</td>
</tr>
<tr>
<td>(ref. 10)</td>
<td>True carbonic acid acidity constant</td>
</tr>
<tr>
<td>(ref. 11)</td>
<td>First carbonic acid dissociation constant</td>
</tr>
<tr>
<td>(ref. 12)</td>
<td>Second carbonic acid dissociation constant</td>
</tr>
<tr>
<td>(ref. 13)</td>
<td>Siderite solubility product constant</td>
</tr>
<tr>
<td>(ref. 14)</td>
<td>Siderite-water O isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 15)</td>
<td>Siderite-water C isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 16)</td>
<td>CO\text{aq}2-\text{aq}2- water O isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 16)</td>
<td>CO\text{aq}2-\text{aq}2- water C isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 17)</td>
<td>CO2\text{aq}2-\text{aq}2- CO2 water O isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 17)</td>
<td>CO2\text{aq}2-\text{aq}2- CO2 water C isotope fractionation factor</td>
</tr>
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<td>(ref. 17)</td>
<td>CO2\text{aq}2-\text{aq}2- CO2 water C isotope fractionation factor</td>
</tr>
<tr>
<td>(ref. 17)</td>
<td>Ice-water O isotope fractionation factor</td>
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<tr>
<td>(ref. 20)</td>
<td>Siderite-water acid digestion O isotope fractionation factor</td>
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5.2 Sensitivity analysis. The model parameters are temperature, $f_{\text{dges}}$, 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 $T = 20 \pm 5$°C, $f_{\text{dges}} = 0.9$, $p_{\text{CO}_2} = 0.6$ 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 $f_{\text{dges}}$—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 continuation 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 $\delta^{18}$O-$\delta^{13}$C covariation to the carbonate mineralogy. The mineralogy affects the chemistry of the aqueous solution (the solubility product constant) and also affects 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 $\alpha_{\text{C-water}}$ 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 eraze the atmospherically derived $\Delta^{18}$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 meteoritic) 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 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$_{\text{SMOW}}$ $\approx$ 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$_{\text{PDB}}$ $\approx$ $-9‰$, 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 $\Delta_{\text{AC}}$ space is non-linear (22). If two end members have $\delta^{18}$O and $\delta^{13}$C that are not sufficiently different (as do the cores and rims of the ALH84001 carbonates), their mixing can yield $\Delta_{47}$ values that are much higher than the end member $\Delta_{47}$ and apparent temperatures that are much lower. For our reliable $\Delta_{47}$ 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 $\Delta_{47}$ 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 $\Delta_{47}$ 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 this 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 second 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 $\Delta_{47}$ equilibrium would yield a spuriously low apparent temperature. However, we digested the carbonate for a total of 12 h at 90°C—long...
enough to extract all of the carbonate based on the dissolution kinetics of carbonates of different cations (23). Indeed, the carbonate yield during the last reaction step (4–12 h) was about a fifth of the first step (0–1 h) and second step (1–4 h). This gives confidence that almost all of the carbonate had been extracted and so even if mixing explains the results of steps 1 and 2, it cannot explain the results of step 3, which are similar to steps 1 and 2. Furthermore, based on the known correlation of $\delta^{18}$O and $\delta^{13}$C with Mg content, preferential dissolution of the end-member compositions requires that magnesian carbonates were extracted early in the digestion, which is discordant with their relative dissolution kinetics.


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**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) $\delta^{13}$C plotted against voltage. (D) $\delta^{18}$O 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 $\delta^{18}$O and $\delta^{13}$C 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 $\delta^{18}$OMOW of 5 and 30‰, and $\delta^{13}$CPDB 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 $f_{d_{gas}}$, but give the maximal increase in pore $pCO_2$ on the lower right of each subfigure. (A) Sensitivity to the value of $f_{d_{gas}}$, 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 $f_{d_{gas}}$. The line styles and colors correspond to the legend in A. A low value of $f_{d_{gas}}$ 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 $\delta^{18}O$, $\delta^{13}C$, and $\Delta_{47}$ measured from the microvolume

<table>
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<tr>
<th>Period</th>
<th>Standard*</th>
<th>Weight, mg</th>
<th>$\Delta \delta^{18}O$, %</th>
<th>$\Delta \delta^{13}C$, %</th>
<th>$\Delta \delta_{47}$, %</th>
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**Correction**

- $\Delta \delta^{18}O$: $-0.092 \pm 0.063 = 0.015$
- $\Delta \delta^{13}C$: $-0.281 \pm 0.072 = 0.039$
- $\Delta \delta_{47}$: $0.159 \pm 0.024 = 0.027$

*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 $D_{47}$ between 0.352 and 0.662%. 95% confidence interval error estimate after application of the correction.

Table S2. All results, including aliquot A

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<thead>
<tr>
<th>Type</th>
<th>$\delta^{18}O$,‰</th>
<th>$\delta^{18}O$,‰</th>
<th>$\delta^{13}C$,‰</th>
<th>$\delta^{13}C$,‰</th>
<th>$\Delta \delta_{47}$,‰</th>
<th>$\Delta \delta_{47}$,‰</th>
<th>$N_{uv}$</th>
<th>Err. 1: stds</th>
<th>Err. 2: stat</th>
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<td>18.13</td>
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<td>10.7</td>
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*The columns from left to right are measurement type (DI = dual-inlet, µV = microvolume), uncorrected $\delta^{18}O$, corrected $\delta^{18}O$ with error (see Table S1), uncorrected $\delta^{13}C$, corrected $\delta^{13}C$ with error, uncorrected $\Delta_{47}$, corrected $\Delta_{47}$, number of acquisitions, $\Delta_{47}$ error estimated from carbonate standards, $\Delta_{47}$ counting statistics error, temperature with error. Details of error estimation are in SI Text section Microvolume Measurements, Standardization, and Error Estimates.