is the APC-LCM complex and peak 3 is an APC trimer). These observations are consistent with a previously described model for chlorosis (23).

Although isolated PC and APC molecules are highly fluorescent, they are difficult to quantify in cells by fluorescence intensities because of the highly efficient energy transfer in the light-harvesting protein complexes, their large spectral overlap, and the fluorescence background from chlorophylls in the photosystems. Our single-cell analysis approach is particularly advantageous because it incorporates chemical separation before fluorescence detection.

We used a chip with three simplified reaction chambers (Fig. 3B) so that up to three cells could be simultaneously lysed. Laser-induced fluorescence detection was used to analyze the –N cells (Fig. 3C) because of their high PBP concentration, whereas molecule counting was used to analyze the –N cells (Fig. 3D). Among the three –N cells shown in Fig. 3D, cell b contains –N cells in peak 2 and 839 copies in peak 3, and the detection limit (two times the standard deviation of background counts from noise) is seven molecules. Figure 3E shows the distribution of the molecule numbers of the two PBS core subassemblies (peaks 2 and 3) from 10 –N cells. The cell-to-cell variation in overall PBP counts of –N cells is much larger than that of +N cells. The number of molecules present in these two subassemblies shows a high correlation ($\rho^2 = 0.93$) over the entire distribution range. This relation indicates that a constant ratio of these two complexes was maintained during the degradation of the PBS under –N conditions and that, as bleaching of the cells proceeded, the complexes were simultaneously lost. These results suggest coordinated degradation of PBS components within the core of the PBS.

Among the 10 –N cells examined, cell a in Fig. 3D had much brighter fluorescence and much higher molecule counts than the others. Its electropherogram resembled those from +N cells, which indicated an incomplete proteolysis of the PBS. This cell represents those –N cells that have atypically bright fluorescence (~5% of all cells) when observed by fluorescence microscopy. In ensemble experiments in which cell populations are examined, these cells would not be detected because of their low frequencies of appearance. The occurrence of this rare cell could be a consequence of genetic variation within the population, although more work (possibly using Synechococcus mutants defective in PBS degradation or using carefully monitored isogenic lines) needs to be done to test this hypothesis.

The method described here should have many applications, making the chemical analysis of single cells by single-molecule counting a new tool for understanding the functioning of cells. Other applications, such as the use of fluorescent proteins that can be genetically fused or the use of dye molecules (such as the biarsenical dye, FlAsH, and its variants) that bind to specific peptide sequences, may further expand the scope of this method to include the monitoring of gene expression and suppression.

References and Notes

Mass-Independent Sulfur Isotopic Compositions in Stratospheric Volcanic Eruptions

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The observed mass-independent sulfur isotopic composition ($\Delta^{33}S$) of volcanic sulfate from the Agung (March 1963) and Pinatubo (June 1991) eruptions recorded in the Antarctic snow provides a mechanism for documenting stratospheric events. The sign of $\Delta^{32}S$ changes over time from an initial positive component to a negative value. $\Delta^{33}S$ is created during photochemical oxidation of sulfur dioxide to sulfuric acid on a monthly time scale, which indicates a fast process. The reproducibility of the results reveals that $\Delta^{33}S$ is a reliable tracer to chemically identify atmospheric processes involved during stratospheric volcanism.

Pinatubo volcanic eruptions dramatically modify climate for several years by injecting large amounts of dust and gases, such as sulfur dioxide (SO$_2$), directly into the stratosphere. Therefore, studying the impact of such eruptions can provide insights into atmospheric chemical sensitivity. The sulfuric acid (H$_2$SO$_4$) layer that formed from SO$_2$ oxidation, within about 1 month for moderate eruptions (1, 2), reflects solar radiation and alters the radiative properties of the atmosphere, cooling the global troposphere (1). Satellite observations (3) or light detection and ranging measurements (4) allow recent volcanic events to be monitored, but the existing volcanic observations database is insufficient to represent all past eruptions (5), and it remains difficult to include volcanic events as a climatic forcing parameter in efforts to understand past climates and to predict the impact of future eruptions. An important step in understanding the climatic impact of volcanic events of different sizes is to identify the chemical processes linked to stratospheric eruptions. During these events, the chemical composition of the atmosphere is strongly perturbed, especially the sulfur budget. The magnitude and character of the perturbation depend on parameters such as eruption strength, sulfur loading, altitude of the volcanic plume, latitude of the volcano, and time of year.

The sulfate concentration of ice cores has proven to be a useful chemical proxy of past vol-

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canism (1), though such records are restricted in interpretative value. Here we report measurements of the mass-independent sulfur isotopic composition of volcanic sulfate, which provide further insights into atmospheric chemical processes associated with stratospheric volcanism.

Mass-dependent isotopic fractionation processes are governed by relative mass differences between the four sulfur isotopes and are described by δ34S ≈ 0.515 δ33S and δ33S ≈ 1.91 δ34S. The deviation from the two mass-dependent relationships is termed mass-independent fractionation (MIF) and quantified by Δ33S and Δ34S (4). Sulfur isotopic anomalies have been found in Archean sulfide and sulfate minerals older than 2 billion years (5, 6) but not in younger rocks, suggesting changes in the sulfur oxidation pathways, which are probably linked to the modification in oxygen abundance and signal the appearance of an ozone layer, and in the shielding ultraviolet (UV) light required to generate the sulfur isotopic anomalies at low altitudes (5–7). A wavelength (λ) dependency of the sulfur MIF has been postulated (5), and SO2 photolysis experiments conducted at different λ have been used to argue this hypothesis (8, 9); but once again, such a view has been recently challenged by new geochemical observations (10), although these observations do not formally rule out the shielding UV-light hypothesis. The sulfur isotopic anomaly is generated by UV light that is shorter than λ = 310 nm, which is only available in the stratosphere in the present atmosphere. Sulfur MIF has been reported in tropospheric aerosols (11) and attributed to stratospheric input of sulfate into the troposphere. Stratospheric volcanic eruptions offer the opportunity to study sulfur MIF under natural atmospheric conditions because large quantities of SO2 are directly injected into the stratosphere and the sulfate is deposited and stably preserved in Antarctic snow. The volcanic signals in ice core records of the stratospheric eruptions of Pinatubo (1991) and an unknown event (1259) exhibit substantial sulfur isotopic anomalies, contrary to the high-tropospheric Cerro Hudson event (1991) (12, 13).

The mechanism responsible for the sulfur isotopic anomaly formation is still under discussion. A recent model study (14) predicts a change in the sign of Δ34S over time, which provides a testable theoretical framework for production of the isotopic anomalies from volcanic events.

Sulfate backgrounds and sulfate deposited on the Antarctic ice sheet by the Agung (8°S, 115°E) and Pinatubo (15°N, 120°E) eruptions in March 1963 and June 1991, respectively, were extracted from snow layers sampled at a high time resolution in a snow pit located at Dome C (75°S, 123°E) and were analyzed for their sulfur isotopic content (15). In nonvolcanic conditions, the sulfate deposited in Dome C snow is predominantly biogenic (16) and was formed in the troposphere. The backgrounds analyzed in this work confirm that sulfate that was formed out of the stratosphere (i.e., away from photochemical oxidations) was free of any sulfur isotopic anomalies [Δ33S = 0 per mil (‰)] (Figs. 1 and 2).

On the other hand, the Agung and Pinatubo volcanic sulfate coming from the stratosphere exhibit large sulfur MIF. In both cases, the maximum sulfur isotopic anomaly occurs before the maximum sulfate concentration (Fig. 1). We observed a stable Δ34S for about a half year, ~1‰ (corrected for background values; table S2), which was followed by a decreasing anomaly, passing through zero% about 1 year after the beginning of the sulfate deposition to reach a bottom value close to ~1‰ at the end of deposition. δ34S values for both Agung and Pinatubo events have an identical range (Fig. 2). The decrease of Δ33S from positive to negative values is accompanied by a significant decrease of δ34S over time.

During the beginning of the Pinatubo eruption, the Δ33S and δ34S values (Fig. 2) are low with respect to the Agung profile. These values are the result of a mass-dependent process caused by the input from the Cerro Hudson (46°S, 73°W) eruption. This volcano erupted in August 1991 and injected 2 Tg of SO2 up to the upper troposphere–lower stratosphere region (13) (that is, below an altitude of 14 km). At this altitude, the lack of UV radiation shorter than λ = 310 nm does not allow SO2 photolysis, which is responsible for the generation of the sulfur isotopic anomaly (8). Measurements of the Cerro Hudson sulfate recorded in snow at the South Pole site (90°S, 120°W), where snow accumulation (~20 cm year−1) permits analysis of the two volcanic events independently, have confirmed the sulfur mass-dependent nature of the Cerro Hudson sulfate, which exhibits a near-zero Δ33S and a δ34S of +5.60‰ (12). Because of its geographic location, the Cerro Hudson plume reached Antarctica before that of Pinatubo (17, 18). The two volcanic signals are not separated in Dome C snow because of the low snow-accumulation rate (~10 cm year−1) at this site. Consequently, the Δ33S and δ34S values (+0.38 and +10.37‰, respectively) correspond to the mass-independent Pinatubo signal that was diluted by the mass-dependent Cerro Hudson contribution.

The mean sulfur isotopic composition of sulfate recorded in the snow during volcanic deposition was estimated with the use of a weighted average. The mean values of δ34S and Δ33S are +9.57 and +0.70‰ for Agung and +9.29 and +0.29% for Pinatubo. For the Pinatubo record, the point at 135 cm is not considered in the weighted average because of the mass-dependent nature of the Cerro Hudson contribution. These calculations allow for the comparison with measurements obtained at the South Pole (12, 19). The Agung Δ33S mean signal measured at the South Pole (19) is estimated to be 2.7 ± 1.1‰, a low value as compared to our calculation. This difference is difficult to explain, but the Pinatubo record can provide some elements for evaluation. The Pinatubo δ34S and Δ33S values at Dome C are lower than the ones measured at the South Pole (+10.89 and +0.67‰, respectively) (12). The South Pole measurements were made only during the first year of sulfate deposition when the isotopic anomaly is positive and the sulfur isotopic composition is enriched in heavy isotopes as compared to the end of deposition. Consequently, fractional analysis of the South Pole Pinatubo record can give higher values as compared to what they would be if the total sulfate deposition was analyzed. Restricting our calculation of the weighted mean to the same chronology, we obtain 13.12 and 0.69‰ for the δ34S and Δ33S values, respectively, which is consistent with the South Pole measurements.

Lithospheric volcanic sulfur does not have a sulfur isotopic anomaly; thus, the isotopic budget requires a mass-weighted positive Δ33S created in the stratosphere to be balanced by a mass-weighted negative Δ33S. We observe a change in the sign of Δ33S, but the budget for both eruptions is significantly different than 0, probably because a portion of the sulfate is lost before reaching Antarctica. The signal loss preferentially affects
The isotopic evolution of the volcanic sulfate is substantial. For the Agung record, the $\delta^{33}S$ fractionation attains 17.5‰ between the beginning and end of the eruption. Such fractionation was observed in stratospheric aerosols at 40°S after the Agung eruption (20). The aerosol measurements were reported from October 1962 to August 1971, but we focused on the period covering the Agung eruption (May 1963 to June 1965). In May 1963, the Agung eruption was complete, and nearly all SO2 emitted had already been oxidized to H2SO4. The slopes of $\delta^{33}S$ plotted as a function of time (Fig. 3) show that the isotopic fractionation was more important at an altitude of 18.3 km than at 15.2 km, which suggests a gradient in isotopic composition with altitude. The similarity between the slopes representing stratospheric aerosols at 15.2-km altitude and sulfate deposited in the Dome C snow makes us think that transport from the tropopause to the ground and depositional effects do not change the $\delta^{33}S$ notably. The sulfate was measured at different latitudes (40°S for stratospheric aerosols and 75°S for snow samples), which suggests that no substantial change in the sulfur isotopic composition occurs during the transport into the stratosphere. The fractionation that is observed as a function of time was attributed to an inverse kinetic-isotope effect, where $34^{\text{SO}_2} + \text{OH}$ reacts faster than $32^{\text{SO}_2} + \text{OH}$, producing enriched $34^{\text{S}}$ sulfate first, followed by gradually depleted aerosols over time (20, 21). This process is mass dependent and alters $\delta^{33}S$ but not $\Delta^{34}S$ in the range of our observed values, even if individual processes may have a slightly different mass-dependent exponent. Because $\delta^{34}S$ does not appear to undergo fractionation during transport, we may extrapolate this statement to the isotopic sulfur anomaly because $\Delta^{33}S$ and $\delta^{34}S$ are linearly correlated (Fig. 2). Consequently, H$_2$SO$_4$ does not appear to undergo any process that alters its sulfur isotopic composition during transport and subsequently its mass-independent composition. The most plausible reaction scenario is that the sulfur isotopic anomaly is generated from SO2 before its oxidation to H$_2$SO$_4$.

Sulfur dioxide photodissociation below 220 nm [($\text{SO}_2 + h\nu = \text{SO} + \text{O}$), where $h\nu$ is photonic energy] accounts for the sulfur MIF in the Archean anoxic atmosphere (7, 14) but not in today’s atmosphere because sulfur monoxide (SO) rapidly reacts with O$_2$ to re-produce SO$_2$ (12, 14). Sulfur dioxide photooxidation ($\text{SO}_2^* + \text{SO}_2 \rightarrow \text{SO}_3 + \text{SO}_2$) has been proposed as a mechanism to generate the observed sulfur isotopic anomaly (12). Excited SO$_2$ can react with ground-state SO$_2$ when its number densities are anomalously high, such as in the first weeks subsequent to a massive volcanic eruption. This reaction may occur at lower altitudes of the stratosphere as compared to SO2 photodissociation if the SO2 plume remains optically thin in the UV window (260 < $\lambda$ < 340 nm). Laboratory experiments have also confirmed the ability of photolysis at these wavelengths to produce sulfur MIF species (8, 9). The creation of two reservoirs composed of SO$_2$ and sulfur trioxide (SO$_3$), which can carry sulfur MIF with the opposite sign, is the requisite condition to generate a $\Delta^{33}S \neq 0$‰ in the volcanic sulfate. According to laboratory experiments conducted at $\lambda > 220$ nm (8, 9), residual SO$_2$ has a $\Delta^{33}S < 0$‰, and produced sulfate (probably via SO$_3$) has a $\Delta^{33}S > 0$‰. Because, in the atmosphere, the reaction to produce H$_2$SO$_4$ between SO$_3$ and H$_2$O is faster than that between SO$_2$ and OH, it is anticipated that sulfate will initially carry a positive $\Delta^{33}S$ and will later become negative as a result of material-balance considerations. Such a view is consistent with the field observations. Recently, SO$_2$ photooxidation was questioned, and SO$_2$ photolysis ($\text{SO}_2 \rightarrow \text{SO}_2 + \text{O}$) was invoked to explain sulfur MIF (14). As in the case of SO2 photooxidation, SO$_3$ photolysis may occur at low altitudes in the stratosphere (195 < $\lambda$ < 300 nm) and generate two reservoirs. Based on this hypothesis, it was suggested that the Pinatubo event should produce a change in the sign of the isotopic anomaly of sulfate but with the negative component reaching the ground first (14), therefore forcing the near-zero negative $\Delta^{33}S$ of Cerro Hudson to be the beginning of the Pinatubo event, in contradiction with the present isotopic observations and concentration measurements (17). SO$_3$ photolysis dominates at altitudes above 37 km (22). Below this altitude, the reaction between SO$_2$ and H$_2$O is faster, and SO$_3$ photolysis is a minor process in the production of a substantial amount of sulfate. SO$_2$ photodissociation remains the leading candidate to account for sulfur MIF of volcanic sulfate. SO$_2$ photodissociation as the source of sulfur MIF is in accordance with SO2 photolysis.
experiments (8, 9). The Agung data are also consistent with laboratory experiments because the $\Delta^{33}$S versus $\delta^{34}$S Agung slope (Fig. 2) is the same as that of the Xe lamp experiment obtained for $\lambda > 220$ nm and very close to that of the KrF laser experiments conducted at 248 nm (8, 9).

The sulfur isotopic anomalies in volcanic samples are much smaller than those observed in Archean rocks older than 2.45 billion years (5, 6, 23, 24). In today’s atmosphere, OH radicals remain the main sink of SO$_2$ emitted after a volcanic eruption, and the SO$_2^* +$ SO$_2$ reaction is a minor reaction when compared to the SO$_2 +$ OH reaction. The sulfur MIF measured in volcanic sulfate recorded in snow is a diluted signal and may actually reach the extreme values recorded in Archean rocks. To estimate the upper limit of the sulfur isotopic anomaly generated by the photooxidation process, researchers should compare the kinetics of the SO$_2 +$ OH and SO$_2^* +$ SO$_2$ reactions. Unfortunately, the rate of SO$_2^* +$ SO$_2$ is controversial (25) and is needed for such quantification.

Sulfur mass-independent composition of volcanic sulfate is a time-dependent process, first displaying a positive $\Delta^{33}$S followed by a negative $\Delta^{33}$S at the end of the volcanic plume depositional process. This process occurs on a monthly time scale before SO$_2$ is fully oxidized in H$_2$SO$_4$, indicating a rapid process. The nonzero average $\Delta^{33}$S observed for the full duration of the event requires two conditions: First, the process creates two reservoirs of MIF with opposing signs; second, these two reservoirs must be physically separated in space and time in addition to having a difference in depositional rates. The only way to explain the oscillation of the $\Delta^{33}$S sign is to consider the fundamental role of aerosols and sedimentation in preserving the isotopic signal. Microphysical processes must be taken into account in models to reproduce sulfur MIF of stratospheric volcanic sulfur. When the relationship between aerosol and sulfur MIF is established, volcanic plume transport may be understood, allowing a precise glaciological record of the climatic impact of stratospheric eruptions.

References and Notes

4. The deviation from the mass-dependent relationships is calculated by the following equations: $\Delta^{33}$S = $\delta^{33}$S – 1000[(1 + $\delta^{34}$S/1000)$^{0.25}$ – 1] and $\Delta^{34}$S = $\delta^{34}$S – 1000[(1 + $\delta^{34}$S/1000)$^{0.25}$ – 1]. Considering the small size of the samples, our analytical accuracy, with a 2σ uncertainty, is equal to 0.12‰ for $\Delta^{33}$S and varies from 0.64 to 1.63‰ for $\Delta^{34}$S. Only $\Delta^{34}$S > 0.12‰ and $\Delta^{34}$S > 0.12‰ are considered as diagnostic of MIF in the present study. Uncertainties (2σ) are 0.07, 0.19, and 0.53 to 1.59‰ for $\Delta^{33}$S, $\Delta^{34}$S, and $\Delta^{34}$S, respectively.
15. Materials and methods are available as supporting material on Science Online.

CO$_2$-Forced Climate and Vegetation Instability During Late Paleozoic Deglaciation

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The late Paleozoic deglaciation is the vegetated Earth’s only recorded icehouse-to-greenhouse transition, yet the climate dynamics remain enigmatic. By using the stable isotopic compositions of soil-formed minerals, fossil-plant matter, and shallow-water brachiopods, we estimated atmospheric partial pressure of carbon dioxide ($p$CO$_2$) and tropical marine surface temperatures during this climate transition. Comparison to southern Gondwanan glacial records documents covariance between inferred shifts in $p$CO$_2$, temperature, and ice volume consistent with greenhouse gas forcing of climate. Major restructuring of paleotropical flora in western Europe occurred in step with climate and $p$CO$_2$ shifts, illustrating the biotic impact associated with past CO$_2$-forced turnover to a permanent ice-free world.

A decade of studying Pleistocene ice cores has unequivocally documented a strong coupling of atmospheric partial pressure of CO$_2$ ($p$CO$_2$) and surface temperatures with changing global ice volume (1, 2). Although the precise mechanistic link between atmospheric greenhouse gases and climate is debated, there remains little doubt that high concentrations of atmospheric CO$_2$ have strongly amplified Earth’s past climates. Anthropogenic CO$_2$ emissions have increased atmospheric CO$_2$ to concentrations higher than at any time in at least the past 650,000 years and could increase it to more than 2000 parts per million by volume (ppmv) as accessible fossil fuel reservoirs are exhausted (3). The last time such concentrations were seen on Earth was at the onset of our modern icehouse [ ~40 to 34 million years ago (Ma)], a transition from ice-free to glacial conditions characterized by repeated C cycle perturba-

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Supporting Online Material

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SOM Text
Fig. S1
Tables S1 and S2
References
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