Reports

Relationship Between the Oxygen Isotope Ratios of Terrestrial Plant Cellulose, Carbon Dioxide, and Water

Abstract. *The ratios of oxygen-18 to oxygen-16 ($^{18}O/^{16}O$) of cellulose purified from two sets of wheat plants grown under conditions similar in all respects except for a large difference in the $^{18}O/^{16}O$ ratios of the carbon dioxide supplied to them differ by only a small amount. The difference in the $^{18}O/^{16}O$ ratios of the cellulose is similar to that observed for the $^{18}O/^{16}O$ ratios of the water present in the plants. These results indicate that the oxygen derived from carbon dioxide undergoes complete exchange with the oxygen of the water in the plant during the synthesis of cellulose and that the $^{18}O/^{16}O$ ratio of the water inside the plant is the primary influence on the $^{18}O/^{16}O$ ratio of cellulose in terrestrial plants.*

The interpretation of variations observed in the $^{18}O/^{16}O$ ratios of cellulose from terrestrial plants (*1*, *2*) has been hindered by a lack of information on the source and isotopic history of the oxygen fixed during photosynthesis. Several models have been proposed to account for the oxygen isotopic composition of cellulose from trees and other plants. These models are based on the assumptions that the oxygen of cellulose is derived either solely from CO_2 (*1*) or from the oxygen atoms of CO_2 and H_2O in the ratio 2:1 (*2*). The possibility of isotopic exchange between the oxygen atoms of CO_2 and H_2O prior to photosynthetic fixation has also been recognized. It has been argued that this exchange involves either meteoric (ground) water (*1*) or water in the plant (*2*). It is unlikely that one can distinguish among these possibilities by analyzing plants that grew in the field.

We undertook the laboratory experiments reported here to determine the relationships between the $^{18}O/^{16}O$ ratio of cellulose and the $^{18}O/^{16}O$ ratios of the CO_2 and H_2O available to terrestrial plants. The results of these experiments have implications for the interpretation of variations in the $^{18}O/^{16}O$ ratios of cellulose as a climatic indicator and for the design of experiments intended to elucidate the path of oxygen in photosynthesis.

Wheat plants were grown from seed in the Plexiglas box shown in Fig. 1. The use of the box made it possible to control the isotopic composition of the H_2O and CO_2 supplied to the plants. Two sets of plants were grown under conditions similar in all respects except for a difference in the $^{18}O/^{16}O$ ratios of the CO_2 sources. In the standard experiment, the $^{18}O/^{16}O$ ratio of the CO_2 was similar to that of atmospheric CO_2, whereas in the ^{18}O-enriched experiment the CO_2 was highly enriched in ^{18}O relative to atmospheric CO_2 (*3*, *4*).

We sampled the air and H_2O flowing into and out of the box for isotopic analysis periodically during the course of each experiment. At the end of each growth period, the plants were harvested and water from the shoots of the plants was quantitatively vacuum-distilled and collected for isotopic analysis. Cellulose was purified (*5*, *6*) only from those parts of the plants which grew after the introduction of the corks (Fig. 1), because during this time isotopic exchange between CO_2 and H_2O flowing through the box was minimized.

The $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios of CO_2 in air (*7*) and the D/H ($^2H/^1H$) (*8*) and $^{18}O/^{16}O$ (*9*) ratios of H_2O were determined by established procedures. Methods for the determination of the $^{13}C/^{12}C$ ratios of cellulose after combustion (*7*) and the $^{18}O/^{16}O$ ratios of a cellulose after pyrolysis in a nickel reaction vessel (*2*) have been described. We determined the D/H ratios of the nonexchangeable carbon-bound hydrogen atoms of cellulose by analyzing cellulose nitrate prepared by the direct nitration of cellulose with a nitric acid–acetic anhydride solution (*6*, *10*, *11*). The isotopic ratios are reported as δ values (*12*).

The δD, $\delta^{18}O$, and $\delta^{13}C$ values of the H_2O and CO_2 supplied to the plants in the two experiments are given in Table 1. The only significant difference between the two experiments was in the $\delta^{18}O$ values of the CO_2 (*13*). The $\delta^{18}O$ values of CO_2 changed as the air passed through the box as a result of partial isotopic equilibration with H_2O (*14*, *15*). Since the plants that were analyzed grew randomly throughout the box, the average $\delta^{18}O$ value of the CO_2 available to them in each experiment was between the value determined at the inlet and outlet ports. Thus, the difference between the average $\delta^{18}O$ values of the CO_2 available in the two cases, although not precisely known, was between 127 ± 25 and 979 ± 115 per mil. This average difference is large enough so that any significant isotopic contribution of oxygen from CO_2 to the oxygen of cellulose could be detected.

The results of the isotopic analysis of cellulose purified from the plants grown in the two experiments are also given in Table 1. The hydrogen and carbon isotopic compositions are consistent with the observations that there were no significant differences between the growth conditions of the two experiments. The δD values of the cellulose samples differ from the δD values of the H_2O supplied in each case by about the same amount. This similarity indicates that the effects of evaporative transpiration, which cause the water in the plant to become enriched in the heavy isotopes of hydrogen and oxygen relative to the water supplied to the plant (*2*, *16*), were the same integrated over the course of the two experiments. The $\delta^{13}C$ values of the cellulose samples are more negative than the $\delta^{13}C$ values of the CO_2 sources by about the same amount as has been observed for wheat grown in the field (*3*, *17*). Thus, the CO_2 metabolism of the laboratory-grown plants does not appear to have been affected by the experimental conditions.

The difference between the $\delta^{18}O$ values of cellulose from the plants grown in the two experiments, whose sources of CO_2 differed in $\delta^{18}O$ value by at least 127 ± 25 per mil and possibly by as much as 500 per mil, is only 8.5 per mil (Table 1). This small difference in the $\delta^{18}O$ values of the cellulose samples is similar to the difference between the $\delta^{18}O$ values of the H_2O inside the plants in the two cases. The average difference in $\delta^{18}O$ values of the plant H_2O over the duration of the experiments is not known precisely, but it must be less than the difference (14 per mil) observed for the H_2O samples distilled from the plants at the end of the growth periods (Table 1) (*18*). Since the $\delta^{18}O$ values of the cellulose samples reflect the $\delta^{18}O$ values of H_2O in the plants but not those of the CO_2 sources, we conclude that the oxygen derived from CO_2 has completely exchanged with that of H_2O in the plant by the time cellulose is formed.

These experiments do not permit the identification of the specific steps leading

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to cellulose synthesis during which the oxygen derived from CO_2 is exchanged with that of H_2O in the plant. However, it seems likely that CO_2 undergoes some equilibration with H_2O in the plant, possibly catalyzed by carbonic anhydrase, prior to its fixation into 3-phosphoglyceric acid, the first intermediate formed during photosynthesis in wheat and other plants with the Calvin type (C_3) photosynthetic metabolism (*19*). It is unlikely that any further exchange of the oxygen atoms derived from CO_2 occurs in the subsequent passage of 3-phosphoglyceric acid through the Calvin cycle until the formation of D-glyceraldehyde phosphate (*20*). The oxygen atom of D-glyceraldehyde phosphate that is derived from the oxygen atoms of CO_2 (one of the two is lost, at random, in intermediate steps) might exchange with the oxygen of H_2O by means of hydrate formation (*20*). Subsequent reactions of the Calvin cycle and the steps associated with the polymerization of glucose to form cellulose do not appear to provide any further opportunity for exchange of the oxygen derived from CO_2 (*20*). Analysis of the $^{18}O/^{16}O$ ratios of 3-phosphoglyceric acid and D-glyceraldehyde phosphate isolated from plants grown on CO_2 sources of different $\delta^{18}O$ values would make it possible to determine the extent of oxygen exchange occurring at these two steps in vivo.

Table 1. Values of δD, $\delta^{18}O$, and $\delta^{13}C$ for H_2O, CO_2, and cellulose from the standard and ^{18}O-enriched experiments; N, number of measurements; N.D., not determined.

Component	δD_{SMOW} (per mil)	$\delta^{18}O_{SMOW}$ (per mil)	$\delta^{13}C_{PDB}$ (per mil)
	Standard experiment		
Inlet CO_2		+ 6.5 ± 0.4 (N = 6)	−39.6 ± 0.3 (N=6)
Outlet CO_2		+33.3 ± 2.5 (N = 3)	−39.4 ± 0.8 (N=3)
Inlet H_2O	−52 ± 1 (N=12)	− 8.1 ± 0.2 (N=12)	
Outlet H_2O	−52 ± 1 (N = 4)	− 8.4 ± 0.6 (N = 4)	
H_2O distilled from plants	− 2 (N = 1)	− 0.8 (N = 1)	
Cellulose	−13, −13	+26.8, +27.9	− 57.2, − 56.5
	^{18}O-enriched experiment		
Inlet CO_2		+ 985 ± 115 (N = 6)	−37.3 ± 0.3 (N=4)
Outlet CO_2		+ 160 ± 25 (N = 3)	N.D.
Inlet H_2O	−52 ± 1 (N=10)	− 8.0 ± 0.3 (N=10)	
Outlet H_2O	−51 ± 0 (N = 4)	− 8.3 ± 0.3 (N = 4)	
H_2O distilled from plants	− 7 (N = 1)	+13.2 (N = 1)	
Cellulose	−16, −15	+35.4, +36.1	− 53.6, − 52.7

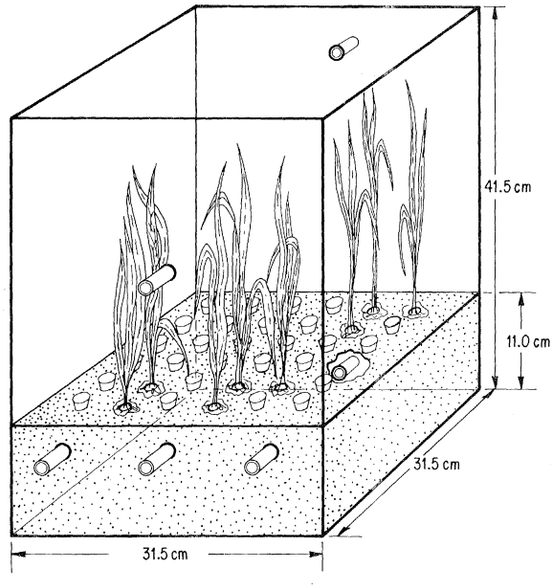

Fig. 1. Diagram of the Plexiglas box used to grow wheat plants under controlled environmental conditions with CO_2 and H_2O of known isotopic composition. The diameter of the holes in the perforated plate is 0.6 cm. The lower compartment of the box has inlet and outlet ports for H_2O and CO_2-free air; the upper compartment has inlet and outlet ports for air containing the average atmospheric concentration of CO_2. The parts of the box stippled are actually opaque. The plants were grown according to the following procedure. Wheat seeds were sprinkled over the surface of a 5-cm bed of Sponge-Rok (baked perlite) in the lower compartment of the box, then covered with a 2.5-cm layer of Sponge-Rok. After the Sponge-Rok had been wetted with distilled H_2O, the perforated plate and the top of the box, both of which are rimmed with gaskets, were sealed into place. During the dark germination period which followed, distilled H_2O was circulated through the box at 1 ml min^{-1} and dry CO_2-free air (scrubbed of CO_2 by passage over Ascarite) was circulated at 470 ml min^{-1}. Four days after the seeds had been sown, the lights (four GTE Sylvania Gro-Lux bulbs located 6 cm above the top of the box) were turned on. The distilled H_2O was replaced by a dilute nutrient solution (*21*), which was supplied at 1 ml min^{-1}. The dry CO_2-free air flow was maintained at 470 ml min^{-1}, and dry air, made by adding CO_2 to dry CO_2-free air to a final concentration of 0.033 percent, was admitted to the upper compartment of the box at 700 ml min^{-1}. All flows were monitored and maintained at the specified rates throughout the remainder of the experiment. Ten days after the seeds had been sown, those holes which did not have seedlings growing through them were plugged with corks in order to reduce isotopic exchange between CO_2 and H_2O flowing through the box. Except for the initial 4 days of darkness, the lights were left on continuously. The temperature was maintained at 25° ± 1°C throughout the experiment. No attempt was made to control the humidity in the box. Relative humidity values when the lights were turned on were on the order of 30 percent and rose gradually, as a result of increased transpiration from the increasing biomass of plants, to a value of 100 percent at the end of the experiments. Plants were harvested 37 days after the seeds had been sown.

Unless there are some major differences in the conditions under which plants grow in the field as compared with those in the experiments discussed here, two of the models that have been advanced to explain variations of terrestrial plant cellulose $^{18}O/^{16}O$ ratios must be discarded. In the first model (*2*) it was postulated that atmospheric CO_2 is fixed into 3-phosphoglyceric acid without undergoing any isotopic equilibration; in the second model (*1*) it was assumed that, if any exchange does occur, it is with meteoric H_2O rather than with H_2O in the plant. The results of this study are compatible with the major assumption of one other model (*2*), which postulated that cellulose oxygen is derived from the oxygen of CO_2 and H_2O inside the plant which are in isotopic equilibrium. In order for this mechanism to hold, however, it is necessary that the $^{18}O/^{16}O$ ratio of cellulose not be affected by isotopic exchange occurring after CO_2 is fixed into 3-phosphoglyceric acid.

The difference between the $\delta^{18}O$ values of cellulose and the H_2O distilled from the plants in the standard experiment, +28.2 per mil, is similar to the difference between the $\delta^{18}O$ values of cellulose from aquatic plants and the H_2O in which they grew in natural environments (*2*). This similarity suggests that the steps that determine the difference between the $\delta^{18}O$ values of cellulose and the H_2O used by a plant are similar in aquatic and terrestrial plants. This difference of +28.2 per mil is also close to what would be expected if the oxygen of cellulose were derived from the oxygen of CO_2 and H_2O in isotopic equilibrium with one another (*14*), in the ratio 2:1 (*2*). Nevertheless, since the $\delta^{18}O$ value of cellulose is determined largely by the $\delta^{18}O$ value of the H_2O inside the plant, the labeling of CO_2 and H_2O with ^{18}O cannot

be used to determine their relative contributions to the oxygen fixed into cellulose.

The conclusion that the oxygen derived from CO_2 equilibrates with the oxygen of H_2O in the plant during the synthesis of cellulose indicates that the $\delta^{18}O$ value of cellulose is primarily a function of the oxygen isotopic composition of the H_2O in the plant. In similar fashion, the δD value of cellulose must be determined largely by the hydrogen isotopic composition of H_2O in the plant (*11*). Starting from the δD and $\delta^{18}O$ values of cellulose, the isotopic composition of the H_2O in a plant at the time the cellulose was formed can be reconstructed if the isotopic fractionations that occur during cellulose synthesis are known. It should also be possible to obtain a measure of the isotopic composition of the meteoric H_2O available to a plant from the values estimated for the isotopic composition of the H_2O in the plant, once the isotopic fractionations that occur during H_2O uptake are defined. The isotopic composition of meteoric H_2O can then be interpreted in terms of climatic temperature (*8, 9*), whereas the difference between the isotopic composition of meteoric H_2O and that of plant H_2O is due to the effects of evaporative transpiration and thus can serve as a measure of the humidity conditions under which the plant grew (*2, 16*). Realization of the full potential of isotopic analysis of cellulose for the purpose of climatic reconstruction requires a thorough understanding of the physical and chemical processes that influence its isotopic composition.

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12. $$\delta^*X = \left[\frac{(^*X/X)_{sample}}{(^*X/X)_{standard}} - 1\right] \times 1000$$
where *X and X are, respectively, the heavier and lighter isotopes of the element. The standards are standard mean ocean water (SMOW) for hydrogen and oxygen and the Peedee belemnite (PDB) carbonate for carbon.
13. The large standard deviations in the $\delta^{18}O$ values of the CO_2 used in the ^{18}O-enriched experiment are caused in part by the necessity of diluting these samples with large amounts of CO_2 of lower ^{18}O concentration before analyzing them in a mass spectrometer routinely used to measure $\delta^{18}O$ values of CO_2 samples of natural isotopic abundance.
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15. There were three sources of H_2O within the box with which CO_2 could have exchanged. These were (i) the H_2O flowing through the box; (ii) the H_2O transpired by the plants and condensed out in the upper compartment of the box; and (iii) the H_2O in the plants, which could exchange with metabolic CO_2 that would subsequently be given off by the plants during respiration and photorespiration. In order to determine the extent of exchange between CO_2 and the H_2O flowing through the box, control experiments were carried out in which all flow conditions through the box were normal but neither plants nor corks were present. The $\delta^{18}O$ values of CO_2 in the air flowing into and out of the box during these control experiments were 916 ± 18 per mil ($N = 4$) and 573 ± 19 per mil ($N = 3$). Under the conditions of the growth experiments, with roughly half the holes in the perforated plate plugged with corks and the rest partially blocked with the shoots of plants (Fig. 1), the amount of this exchange would be reduced. The extent of exchange of CO_2 with H_2O condensed in the upper compartment of the box would increase during the course of the experiments, as the amount of H_2O transpired by the plants increased. In similar fashion, the amount of CO_2 given off during respiration and photorespiration would also increase during the course of the experiments, as the biomass of the plants increased. The combination of increases in these last two effects accounts for the observation that the $\delta^{18}O$ values of CO_2 in the air flowing out of the box, collected weekly during the last 3 weeks of each experiment, showed progressively larger effects of exchange. The $\delta^{18}O$ values for this CO_2 for the standard experiment were +30.6, +33.7, and +35.6 per mil; the corresponding values for the ^{18}O-enriched experiment were +186, +159, and +136 per mil.
The $\delta^{18}O$ values of the water flowing into and out of the box were virtually identical in each experiment. The oxygen isotopic composition of the H_2O supply was not affected by equilibration between CO_2 and H_2O because of the large ratio of H_2O to CO_2 in the box at any given time.
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18. The difference between the $\delta^{18}O$ values of H_2O distilled from the plants in the two experiments is due to isotopic exchange between oxygen of the H_2O and oxygen derived from the two CO_2 sources of markedly different ^{18}O contents. The value of 14.0 per mil represents an upper limit on the difference between $\delta^{18}O$ values of the plant H_2O, since the H_2O samples on which the measurements were made were collected when the amount of exchange was greater (since the relative humidity was higher, the transpiration rate was lower and thus the residence time of H_2O in the plants was longer) than at any other time during the experiments.
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22. We thank C. Kendall and S. N. Kurisu for technical assistance and R. H. Becker, J. Bonner, W. H. Klein, and R. M. Potter for discussions. This study was supported by NSF grant ATM76-03972. Contribution 3130 of the Division of Geological and Planetary Sciences, California Institute of Technology.

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The Moon: Sources of the Crustal Magnetic Anomalies

Abstract. *Previously unmapped Apollo 16 subsatellite magnetometer data collected at low altitudes over the lunar near side are presented. Medium-amplitude magnetic anomalies exist over the Fra Mauro and Cayley Formations (primary and secondary basin ejecta emplaced 3.8 to 4.0 billion years ago) but are nearly absent over the maria and over the craters Copernicus, Kepler, and Reiner and their encircling ejecta mantles. The largest observed anomaly (radial component ~ 21 gammas at an altitude of 20 kilometers) is exactly correlated with a conspicuous light-colored deposit on western Oceanus Procellarum known as Reiner γ. Assuming that the Reiner γ deposit is the source body and estimating its maximum average thickness as 10 meters, a minimum mean magnetization level of 5.2 ± 2.4 × 10^{-2} electromagnetic units per gram, or ~ 500 times the stable magnetization component of the most magnetic returned sample, is calculated. An age for its emplacement of ≤ 2.9 billion years is inferred from photogeologic evidence, implying that magnetization of lunar crustal materials must have continued for a period exceeding 1 billion years.*

A fundamental issue in the interpretation of lunar magnetism has been the identity of those the crustal materials that are magnetized to sufficient levels to produce the orbital anomalies (*1*). Previous investigations of these anomalies, using Apollo 15 and Apollo 16 subsatellite magnetometer data, were restricted to the 3 or 4 days per month when the moon was in the magnetically quiet environment of the geomagnetic tail lobes (*2, 3*). Because of several unfortunate dynamical commensurabilities (including the equality of the lunar orbital and spin periods), the subsatellites always traversed nearly identical surface locations on successive lunations and useful selenographic coverage was limited to narrow bands at a minimum altitude of 65 km across the heavily cratered farside highlands. No coverage at low altitudes across the geologically well understood near side was available.

The lack of nearside maps and the proximity of many farside anomalies to large craters such as Van de Graaff led to early suspicions that cratering itself could be an important mechanism for the

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