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NOTE

Biogeochemical implications of the isotopic equilibrium fractionation factor between the oxygen atoms of acetone and water

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Abstract—Carbonyl oxygens of organic molecules undergo isotopic exchange with water during reversible hydration reactions. The equilibrium isotopic fractionation factors between the carbonyl oxygen of acetone and water at 15°, 25°, and 35°C are 1.028, 1.028, and 1.026 respectively. The differences between the $\delta^{18}O$ values of the carbonyl oxygen of acetone and of the water with which it is in equilibrium are similar to the differences that have been observed between the $\delta^{18}O$ values of cellulose and the water used in its synthesis by a variety of aquatic plants and animals. Additionally, the identity of the acetone-water fractionation factors at 15° and 25°C parallels the observation that the difference between the $\delta^{18}O$ values of cellulose and water shows no temperature dependence for individual species of plants grown over the same temperature range. These results are discussed in relation to the proposal that the oxygen isotopic relationship between cellulose and water is established by isotopic exchange occurring during the hydration of carbonyl groups of the intermediates of cellulose synthesis.

INTRODUCTION

THE RELATIONSHIP between the $\delta^{18}O$ values of cellulose and the water at the site of its synthesis is relatively constant. This relationship can be expressed in terms of $\alpha^*_{\text{CELLULOSE-H}_2\text{O}}$ values, where

$$\alpha^*_{\text{CELLULOSE-H}_2\text{O}} = \frac{(^{18}O/^{16}O)_{\text{CELLULOSE}}}{(^{18}O/^{16}O)_{H_2O}} = \frac{\delta^{18}O_{\text{CELLULOSE}} + 1000‰}{\delta^{18}O_{H_2O} + 1000‰}$$

(We use an asterisk to indicate that thermodynamic equilibrium is not implied by this ratio.) Results of studies in which $\alpha^*_{\text{CELLULOSE-H}_2\text{O}}$ values of aquatic and terrestrial plants and animals were determined are shown in Fig. 1. In all cases, the $\alpha^*_{\text{CELLULOSE-H}_2\text{O}}$ values obtained were between 1.025 and 1.030. In addition, 3 species of freshwater vascular plants grown at 15°C, 20°C and/or 25°C did not show any significant difference in $\alpha^*_{\text{CELLULOSE-H}_2\text{O}}$ values (Fig. 1).

Several models have been advanced to explain the relationship between the $\delta^{18}O$ values of cellulose and the water used in its synthesis. One of these, which involved the assumption that CO_2 equilibrates with meteoric water prior to uptake by plants (GRAY and THOMPSON, 1976), was eliminated by the observation that the $\delta^{18}O$ value of cellulose is not influenced by the $\delta^{18}O$ value of atmospheric CO_2 (DENIRO and EPSTEIN, 1979). The other models involve two types of isotopic exchange reactions occurring after CO_2 uptake. The first type involves partial or complete equilibration of CO_2 with leaf water prior to CO_2 fixation into 3-phosphoglyceric acid, the first intermediate of the Calvin cycle (EPSTEIN *et al.*, 1977). The second involves isotopic exchange during hydration of carbonyl groups in the intermediates of the Calvin cycle and of cellulose synthesis (DENIRO and EPSTEIN, 1981). DENIRO and EPSTEIN (1981) discussed both of these models in light of the available data and concluded that isotope exchange during carbonyl hydration reactions probably accounts for the oxygen isotopic relationship between cellulose and water used in its synthesis. However, they noted that this model could not be tested because there were no data on the magnitude and temperature dependence of the isotopic fractionations that occurs during carbonyl hydration reactions.

In this study we present the first measurements of the equilibrium isotopic fractionation factor between the carbonyl oxygen of an organic molecule and the oxygen of water. We also determined the effect of temperature on this fractionation factor over the temperature range in which many plants grow. We relate these observations to the model proposed by DENIRO and EPSTEIN (1981) to account for the oxygen isotopic relationship between cellulose and water.

MATERIALS AND METHODS

We determined the isotopic equilibrium fractionation factor between the oxygen of a carbonyl group and that of water by allowing CO_2, H_2O, and a compound containing a single carbonyl group, symbolized here by RCO, to equilibrate in a closed system. At equilibrium, this system contains a fourth component, the hydrated form of the carbonyl-containing compound, symbolized here by $RC(OH)_2$. This fourth component is generated by the reversible hydration reaction

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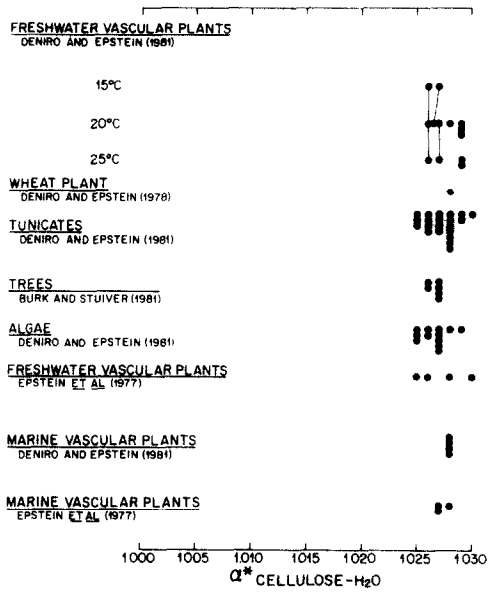

FIG. 1. $\alpha^*_{\text{CELLULOSE-H}_2\text{O}}$ values for aquatic and terrestrial plants and animals (tunicates). The $\alpha^*_{\text{CELLULOSE-H}_2\text{O}}$ values for aquatic plants and animals were calculated using $\delta^{18}O$ values reported for their cellulose and ambient waters, except for the freshwater vascular plants of EPSTEIN *et al.* (1977). EPSTEIN *et al.* (1977) reported the δD values of cellulose nitrate and the $\delta^{18}O$ values of cellulose for these plants. We used the relationship between the δD values of cellulose nitrate and the δD values of ambient waters (EPSTEIN *et al.*, 1976) and the relationship between the δD values and $\delta^{18}O$ values of meteoric waters (CRAIG, 1961) to estimate the $\delta^{18}O$ values of the ambient waters these plants used. In the case of aquatic plants grown at different temperatures (DENIRO and EPSTEIN, 1981), points from the same species are connected by a line. The $\alpha^*_{\text{CELLULOSE-H}_2\text{O}}$ value for the wheat plant was calculated using $\delta^{18}O$ values reported for leaf water and leaf cellulose. The values for trees were calculated by BURK and STUIVER (1981) using a model discussed in their paper.

$$H_2O + RCO \rightleftharpoons RC(OH)_2$$

The isotopic mass balance expression for this system is given by Eq. (1)

$$n^i_{CO_2}\cdot\delta^i_{CO_2} + n^i_{H_2O}\cdot\delta^i_{H_2O} + n^i_{RCO}\cdot\delta^i_{RCO} = n^f_{CO_2}\cdot\delta^f_{CO_2} + n^f_{H_2O}\cdot\delta^f_{H_2O} + n^f_{RCO}\cdot\delta^f_{RCO} + n^f_{RC(OH)_2}\cdot\delta^f_{RC(OH)_2} \quad (1)$$

where

n_x = moles of component x
δ_x = $\delta^{18}O$ value of component x
i = initial conditions
f = final conditions

Now, since

(a) $n^i_{CO_2} = n^f_{CO_2}$,

because CO_2 does not enter into the reaction chemically, and

(b) $n^f_{H_2O} = n^i_{H_2O} - n^f_{RC(OH)_2}$,

(c) $n^f_{RCO} = n^i_{RCO} - n^f_{RC(OH)_2}$,

because one mole of H_2O reacts with one mole of RCO to give one mole of $RC(OH)_2$, Eqn. (1) can be simplified to Eqn. (2)

$$n^i_{CO_2}\cdot\delta^i_{CO_2} + n^i_{H_2O}\cdot\delta^i_{H_2O} + n^i_{RCO}\cdot\delta^i_{RCO} = n^i_{CO_2}\cdot\delta^f_{CO_2} + (n^i_{H_2O} - n^f_{RC(OH)_2})\cdot\delta^f_{H_2O} + (n^i_{RCO} - n^f_{RC(OH)_2})\cdot\delta^f_{RCO} + n^f_{RC(OH)_2}\cdot\delta^f_{RC(OH)_2} \quad (2)$$

The compound containing a single carbonyl group that we utilized was acetone. At equilibrium, $n^f_{RCO}/n^f_{RC(OH)_2}$ for acetone is 500 (GREENZAID *et al.*, 1967), and thus, Eqns. (b) and (c) above can be approximated as

(d) $n^f_{H_2O} = n^i_{H_2O}$

(e) $n^f_{RCO} = n^i_{RCO}$

Additionally, the term $n^f_{RC(OH)_2}\cdot\delta^f_{RC(OH)_2}$ is negligible compared to other terms on the right side of Eqn. 2. Taking these considerations into account, Eqn. (2) simplifies to Eqn. (3)

$$n^i_{CO_2}\cdot\delta^i_{CO_2} + n^i_{H_2O}\cdot\delta^i_{H_2O} + n^i_{RCO}\cdot\delta^i_{RCO} = n^i_{CO_2}\cdot\delta^f_{CO_2} + n^i_{H_2O}\cdot\delta^f_{H_2O} + n^i_{RCO}\cdot\delta^f_{RCO} \quad (3)$$

The equilibration was carried out in standard water equilibration vessels (EPSTEIN and MAYEDA, 1953) whose volume was about 10 cc. We started the equilibration with about 300 μmoles of CO_2, 55500 μmoles of H_2O, and 13600 μmoles of acetone. The initial $\delta^{18}O$ value of the CO_2 was determined by measuring an aliquot of tank CO_2. The initial $\delta^{18}O$ value of the H_2O was determined by the method of EPSTEIN and MAYEDA (1953). The initial $\delta^{18}O$ value of the carbonyl oxygen of acetone (note that acetone contains only one oxygen atom) was determined by the method of RITTENBURG and PONTICORVO (1956) as modified by BURK (1979).

After equilibrium was attained, we isolated an aliquot of the gas phase. We separated the CO_2 from the acetone and H_2O vapor by passing the gaseous mixture through silica gel. Control experiments showed that the CO_2 passed through the silica gel quantitatively and without isotopic fractionation, while the H_2O and acetone were totally adsorbed on the gel. We then measured the $\delta^{18}O$ of the CO_2 to obtain $\delta^f_{CO_2}$. The value of $\delta^f_{H_2O}$ was calculated from the temperature-corrected isotopic equilibrium relationship between CO_2 and H_2O (BOTTINGA and CRAIG, 1969). We were then able to calculate δ^f_{RCO} using Eqn. (3), since all quantities except δ^f_{RCO} were known. The equilibrium fractionation factor, $\alpha_{RCO\text{-}H_2O}$, was calculated from the relationship

$$\alpha_{RCO\text{-}H_2O} = \frac{(^{18}O/^{16}O)_{RCO}}{(^{18}O/^{16}O)_{H_2O}} = \frac{\delta^f_{RCO} + 1000‰}{\delta^f_{H_2O} + 1000‰}$$

The measurements were carried out at 15°, 25° and 35°C using several waters having different $\delta^{18}O$ values. All measurements reported here were based on two days of equilibration. The half-time for equilibration between acetone and water is about 10 minutes (MODEL *et al.*, 1968) and thus two days is ample time for equilibration to occur. Equilibration reactions which were run over a four day period did not give results that differed significantly from those obtained for two day equilibrations.

RESULTS

The $\alpha_{RCO\text{-}H_2O}$ values calculated from equilibrations done at three temperatures are shown in Fig. 2. There is no significant difference between the mean $\alpha_{RCO\text{-}H_2O}$ values at 15°C and 25°C, both of which were 1.028 (Student's t-test at $P = 0.05$ level). The

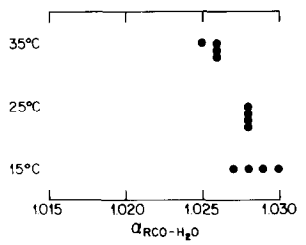

FIG. 2. α_{RCO-H_2O} values for acetone at the indicated temperatures.

mean α_{RCO-H_2O} value at 35°C, 1.026, is significantly different from the values obtained at 15° and at 25°C (Student's t-tests at $P = 0.05$ level).

The α_{RCO-H_2O} values we observed at all three temperatures are similar to the $\alpha^*_{CELLULOSE-H_2O}$ values shown in Fig. 1. The absence of a difference between the α_{RCO-H_2O} values at 15° and 25°C (Fig. 2) parallels the observation of no temperature effect on $\alpha^*_{CELLULOSE-H_2O}$ values for plants grown over the same temperature range (Fig. 1).

DISCUSSION

The proposal that isotopic fractionation during hydration reactions of carbonyl groups of the intermediates of cellulose synthesis accounts for the isotopic relationship between cellulose and water (DENIRO and EPSTEIN, 1981) will be discussed in view of the results of this study.

The pathway from CO_2 fixation to cellulose formation in plants is shown in Fig. 3. All of the oxygen atoms which occur as carbonyl groups in the intermediates of this pathway can undergo hydration reactions, during which isotopic exchange with the oxygen of water would occur. All oxygen entering the photosynthetic path *de novo* (in the form of CO_2 and H_2O) that ends up in cellulose passes through the carbonyl group of 3-phosphoglyceraldehyde. Thus, the isotopic exchange occurring during the hydration of this carbonyl group, illustrated in the upper right corner of Fig. 3, may be the primary influence on the isotopic relationship between cellulose and water.

In the case of tunicates, animals which contain large amounts of cellulose in their body walls, cellulose is probably synthesized *via* the gluconeogenic pathway. The gluconeogenic pathway involves the same intermediates as the pathway leading from 3-phosphoglycerate to glucose 1-phosphate in plants (Fig. 3) (WHITE *et al.*, 1973). Thus the isotopic exchange occurring during hydration of carbonyl groups of intermediates of the pathway shown in Fig. 3 could also establish the isotopic relationship between cellulose and water in tunicates.

The attainment of isotopic equilibrium during carbonyl hydration reactions of the intermediates of cellulose synthesis would establish the constant oxygen isotopic relationship between cellulose and water if three conditions are met. First, the magnitude and temperature dependence of α_{RCO-H_2O} values between water and the carbonyl-containing metabolic intermediates involved in cellulose synthesis must be similar to those we obtained for acetone and water. It is not possible to measure α_{RCO-H_2O} values for metabolic intermediates with the method employed here. Our measurements of the initial $\delta^{18}O$ value of the carbonyl oxygen can be done only for compounds that have the carbonyl oxygen as the sole oxygen. All metabolic intermediates have other oxygens in addition to the carbonyl oxygen. It might be possible to calculate α_{RCO-H_2O} values for the intermediates using spectroscopic data (UREY, 1947). The second condition is that the carbonyl oxygens of the metabolic intermediates must reach isotopic equilibrium faster than they are converted to oxygens in hydroxyl, carboxyl or esters groups, all of which are non-exchangeable under physiological conditions (SAMUEL and SILVER, 1965). The half-time for attaining isotopic equilibrium has been measured for a few in-

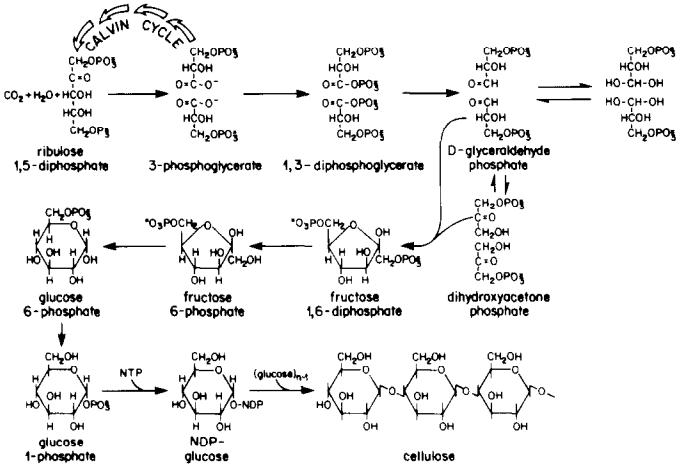

FIG. 3. Pathway from carbon dioxide fixation to cellulose formation in plants. The hydration reaction of the carbonyl oxygen of 3-phosphoglyceraldehyde is shown to the right of figure. Regeneration of ribulose 1,5-diphosphate occurs *via* the Calvin cycle as indicated at the top of the figure.

termediates. The half-times for fructose 1,6-diphosphate, fructose 6-phosphate, and dihydroxyacetone phosphate are 29.5 minutes, 166 minutes, and less than 10 seconds respectively (REYNOLDS *et al.*, 1971; MODEL *et al.*, 1968). Data on exchange rates of other intermediates involved in the Calvin cycle and in cellulose synthesis are not available. Additionally, we are not aware of data that would allow estimation of how long the carbonyl groups in these intermediates would be undergoing exchange in the plant before being converted to non-exchangeable moieties. The final condition which must be met for the model to apply is that there be no isotopic fractionation when the carbonyl oxygens are converted to non-exchangeable hydroxyl, carboxyl or ester oxygens. The oxygen atoms in question are known to stay attached to carbon during a number of these reactions (WHITE *et al.*, 1973; PAGE, 1976). This observation eliminates the possibility of isotopic fractionation that might occur if new oxygen atoms were substituted for those that had already undergone equilibration. However, the possibility of kinetic isotopic effects during the conversion of carbonyl oxygens to non-exchangeable moieties cannot be eliminated.

Experiments designed to test whether or not each of the three conditions discussed above are met must be carried out in order to test the proposal (DENIRO and EPSTEIN, 1981) that the constant isotopic relationship between cellulose and water is established by isotopic equilibrium attained during the hydration of carbonyl groups in the intermediates of cellulose synthesis. The similarily of the magnitude and temperature dependence of the equilibrium isotopic fractionation between the carbonyl oxygen of acetone and the oxygen of water and the magnitude and temperature dependence of the oxygen isotopic relationship between cellulose and the water at the site of its synthesis should serve as the impetus for such experiments.

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