Available online at www.sciencedirect.com

SciVerse ScienceDirect

Geochimica et Cosmochimica Acta 112 (2013) 178–191

Geochimica et Cosmochimica Acta

www.elsevier.com/locate/gca

Position-specific measurement of oxygen isotope ratios in cellulose: Isotopic exchange during heterotrophic cellulose synthesis

John S. Waterhouse [a,*], Shuying Cheng [a,b], Dieter Juchelka [c], Neil J. Loader [b], Danny McCarroll [b], V. Roy Switsur [a], Lata Gautam [a]

[a] Department of Life Sciences, Anglia Ruskin University, East Road, Cambridge CB1 1PT, UK
[b] Department of Geography, College of Science, Swansea University, Swansea SA2 8PP, UK
[c] Thermo Fisher Scientific, Hanna-Kunath Str. 11, 28199 Bremen, Germany

Received 7 May 2012; accepted in revised form 12 February 2013; available online 27 February 2013

Abstract

We describe the first reported method for the measurement of oxygen isotope ratios at each position in the glucose units of the cellulose molecule. The overall process comprises a series of synthetic organic sequences, by which α-cellulose is hydrolysed to glucose, and oxygen atoms at specific positions in the glucose molecule are removed in samples of benzoic acid for measurement of $\delta^{18}O$. Values of $\delta^{18}O$ at specific positions in cellulose are calculated from these $\delta^{18}O$ values and the overall $\delta^{18}O$ value of the cellulose. We apply the method to determine the degree to which oxygen atoms at each position undergo isotopic exchange with water during heterotrophic cellulose synthesis, such as occurs in the cambium of trees. To do this we extract α-cellulose from wheat seedlings germinated in the dark in aqueous media of differing oxygen isotope ratios. Results indicate that oxygen atoms at positions 5 and 6 (O-5 and O-6 respectively) undergo around 80% exchange with medium water, O-3 undergoes around 50% exchange, and O-2 and O-4 do not undergo isotopic exchange. The results have important implications for extracting palaeoclimatic records from oxygen isotope time series obtained from tree ring cellulose. As O-5 and O-6 undergo significant exchange with medium water during heterotrophic cellulose synthesis, oxygen isotopes at these positions in tree ring cellulose should carry a predominantly trunk (source) water signal. On the other hand, O-2 and O-4 should retain the isotopic signature of leaf water in tree ring cellulose. Our method therefore potentially enables the separate reconstruction of past temperature and humidity data from oxygen isotope ratios of tree ring cellulose – something that has hitherto not been possible. The measured degrees of isotopic exchange are to some extent unexpected and cannot be fully explained using current biochemical mechanisms, suggesting that knowledge of these processes is incomplete.

1. INTRODUCTION

Oxygen isotope ratios in the cellulose of living or subfossil plants, and in particular in the annual growth rings of trees, are recognized as a potentially valuable source of information about modern and past climates (McCarroll and Loader, 2004; Daley et al., 2009). The original source of the oxygen atoms in tree rings is precipitation, the oxygen isotope ratio of which is known to depend in part upon temperature (Dansgaard, 1964); however, the route from precipitation to cellulose is by no means direct. It is known that there is no isotopic fractionation when trees take up water (source water) into the trunk (Wershaw et al., 1966); but several factors can complicate the relationship between isotope ratios in precipitation and source water used by the tree. Among these factors are evaporative enrichment of soil water and the sampling of waters of

* Corresponding author.
E-mail address: john.waterhouse@anglia.ac.uk (J.S. Waterhouse).

0016-7037/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.gca.2013.02.021

differing isotopic values at differing depths by tree roots (Dawson and Pate, 1996).

In the leaf, evapotranspiration of water from the stem causes isotopic enrichment of oxygen in water, the degree of which depends primarily upon the ratio of external to internal vapour pressures and upon the isotopic value of air moisture (Dongmann et al., 1974; Flanagan et al., 1991). Under conditions where there is an isotopic steady state in the leaf and source water and when atmospheric water vapour are in isotopic equilibrium, the situation is simplified and relative humidity becomes the major factor controlling isotopic enrichment (Saurer et al., 1997). Other factors also play a role in determining the isotopic content of leaf water; for example the Péclet effect (Farquhar and Lloyd, 1993; Barbour et al., 2000) reduces isotopic enrichment in leaf water. Nevertheless, carbohydrates synthesized in the leaf will have oxygen isotope ratios that record to a certain extent values of relative humidity at the time of photosynthesis. It is known that translocated sugars undergo further isotopic modification during cellulose synthesis in the trunk cambium: approximately 40% of the oxygen atoms of the cellulose molecule undergo isotopic exchange with stem (source) water (Sternberg et al., 1986; Roden et al., 2000). This exchange reinforces the source water isotopic signature in tree ring cellulose, without necessarily removing the humidity signal. The oxygen isotope ratios of cellulose are therefore likely to record a combination of environmental signals arising from precipitation (temperature) and leaf-water enrichment (relative humidity). The degree to which a particular tree records these parameters, however, will depend upon local conditions, such as the degree to which the source water of the tree is related to precipitation (Waterhouse et al., 2002).

The fact that isotope ratios in tree ring cellulose are generally determined by more than one climatic factor presents a major problem for attempts to derive palaeoclimatic information from oxygen isotopes in trees. For example, a high value of $\delta^{18}O$ in tree ring cellulose could reflect an increase in temperature (source water signal) or decrease in relative humidity (leaf water signal) (Sternberg et al., 2003). This complication reduces the power of oxygen isotopes for environmental reconstruction. In cases where significant relationships between tree ring $\delta^{18}O$ and single climatic parameters have been reported (Ramesh et al., 1986; Switsur et al., 1994, 1995; Robertson et al., 2001; Treydte et al., 2006; Loader et al., 2008, 2010), correlation coefficients between tree ring $\delta^{18}O$ and the instrumental climatic data are typically less than 0.65. Such values are generally too low for reliable climatic reconstruction (McCarroll and Pawellek, 2001; Lucy et al., 2008). One solution to this problem would be to separate the source water signal from the leaf signal. Current methods of isotopic analysis of cellulose, however, give a mean value of the individual positions in the glucose ring of cellulose, thus potentially mixing the source water and leaf water isotopic signals. There is no reason to suppose that oxygen isotope ratios at different positions in the glucose ring of cellulose are the same, owing to differences in the biochemical routes leading to their incorporation; hence it is possible that the five oxygen atoms in the glucose ring record source water and leaf water isotope ratios to different extents, thus providing a potential means of separating the two isotopic signatures. In pioneering research, Sternberg et al. (2003) developed a method for determining isotope values of oxygen at position 2 (O-2) of the glucose ring of cellulose (see Scheme 1 for numbering of oxygen atoms). They provided evidence that this oxygen was fully exchanged with water during heterotrophic cellulose synthesis, implying that this oxygen should carry a clear source water signal in tree ring cellulose. In later studies, however, Sternberg et al. (2006, 2007) reassessed the degree of isotopic exchange of O-2 with water, and the expectation that O-2 of tree ring cellulose would record $\delta^{18}O$ of source water was not borne out. Therefore there remains a need (i) to develop a method of measuring the $\delta^{18}O$ values at each position in cellulose and (ii) to determine the degree of isotopic exchange of oxygen at each position during heterotrophic synthesis of cellulose in the trunk. The expectation is that positions at which oxygen atoms fully exchange with trunk water relate more strongly to source water, whereas oxygen atoms that do not exchange relate more strongly to leaf water. This would provide a means of deconvolving the climatic processes controlling isotope ratios in tree ring cellulose.

In this paper we describe the first reported procedure for the determination of the oxygen isotope ratio of each oxygen in the glucose ring of the cellulose molecule. This is a development and extension of the methodology of Mullane et al. (1988), who described a method for measuring the mean isotope ratio of four of the oxygen atoms of cellulose. We apply the procedure to determine the degree of isotopic exchange at each position during heterotrophic cellulose synthesis, for which we use a modified version of the method of Sternberg et al. (2003) for the heterotrophic generation of cellulose from wheat seedlings.

2. THEORY

Scheme 1 shows the cellulose molecule (**1**) as a repeating set of glucose units. Each unit has five separate oxygen atoms numbered 2–6, corresponding to the carbon atoms to which they are bonded. Each of these oxygen atoms will potentially have a different $^{18}O/^{16}O$ ratio, the value of which is represented as δ_n, where n is the positional number of the oxygen atom. The average value of $\delta^{18}O$ for the cellulose molecule (δ_{cell}), over small ranges of δ, can therefore be expressed as:

$$\delta_{cell} = \frac{\delta_2 + \delta_3 + \delta_4 + \delta_5 + \delta_6}{5} \quad (1)$$

Acid hydrolysis of cellulose gives a mixture of α- and β-D-glucose (**2**), in which one of the oxygen atoms (O-1) is derived from the water used in hydrolysis. Schemes 2–5 show in outline how the glucose is derivatized to a series of benzoate esters, in which the benzoate groups contain between one and four of the oxygen atoms (O-2 to O-6) of the original cellulose. (Further explanation of the chemistry involved can be found in Appendix 2.) S_N2-type cleavage of the ester groups using pyridinium chloride (py^+Cl^-) yield samples of benzoic acid, which are represented as BA*n*, where n is the number (2–5) of the Scheme in which they