Phytochemistry 145 (2018) 197–206

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

On the contributions of photorespiration and compartmentation to the contrasting intramolecular ^{2}H profiles of C_3 and C_4 plant sugars

Youping Zhou [a, b, f, *], Benli Zhang [a], Hilary Stuart-Williams [d], Kliti Grice [c], Charles H. Hocart [a, d], Arthur Gessler [b, e], Zachary E. Kayler [b, g], Graham D. Farquhar [d]

[a] School of Chemistry & Chemical Engineering, Shaanxi University of Science & Technology, Xi'an, China
[b] Institute for Landscape Biogeochemistry, ZALF, Germany
[c] WA-Organic and Isotope Geochemistry Centre, Department of Chemistry, Curtin University, Australia
[d] Research School of Biology, Australian National University, Australia
[e] Swiss Federal Institute for Forest, Snow and Landscape Research, Birmensdorf, Switzerland
[f] Leibniz Institute for Freshwater Ecology & Inland Fisheries, Germany
[g] USDA Forest Service, Northern Research Station, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA

ARTICLE INFO

Article history:
Received 28 October 2017
Accepted 3 November 2017

Keywords:
Sugar biosynthesis
Intramolecular ^{2}H distribution
Isotope effect
C_3 photorespiration
C_4 compartmentation

ABSTRACT

Compartmentation of C_4 photosynthetic biochemistry into bundle sheath (BS) and mesophyll (M) cells, and photorespiration in C_3 plants is predicted to have hydrogen isotopic consequences for metabolites at both molecular and site-specific levels. Molecular-level evidence was recently reported (Zhou et al., 2016), but evidence at the site-specific level is still lacking. We propose that such evidence exists in the contrasting ^{2}H distribution profiles of glucose samples from naturally grown C_3, C_4 and CAM plants: photorespiration contributes to the relative ^{2}H enrichment in H^5 and relative ^{2}H depletion in H^1 & H^6 (the average of the two pro-chiral Hs and in particular $H^{6,\ pro\text{-}R}$) in C_3 glucose, while ^{2}H-enriched C_3 mesophyll cellular (chloroplastic) water most likely contributes to the enrichment at H^4; export of (transferable hydrogen atoms of) NADPH from C_4 mesophyll cells to bundle sheath cells (via the malate shuttle) and incorporation of ^{2}H-relatively unenriched BS cellular water contribute to the relative depletion of H^4 & H^5 respectively; shuttling of triose-phosphates (PGA: phosphoglycerate dand DHAP: dihydroacetone phosphate) between C_4 bundle sheath and mesophyll cells contributes to the relative enrichment in H^1 & H^6 (in particular $H^{6,\ pro\text{-}R}$) in C_4 glucose.

1. Introduction

The stable hydrogen isotopic ratio ($^{2}H/^{1}H$) of carbon-bound hydrogen (C-H) of naturally synthesized plant carbohydrates (and other molecules such as lipids) has the potential to provide retrospective information about plant physiology, metabolism and climate (Luo and Sternberg, 1991; Luo et al., 1991; Martin and Martin, 1991; Yakir, 1992; Gleixner and schmidt, 1997; Sessions et al., 1999; Billault et al., 2001; Grice et al., 2008; Robins et al., 2003; 2008; Zhang et al., 2009; Zhou et al., 2010, 2011; 2015, 2016; Ehlers et al., 2015). Notably the $^{2}H/^{1}H$ in natural archives such as tree ring cellulose (Liu et al., 2015) or plant waxes in sediments (Niedermeyer et al., 2016) allows the reconstruction of long-term climate patterns and its impact on plant function. The full potential of such applications however has not been realized as photosynthetic and biosynthetic pathway-specific differences in $^{2}H/^{1}H$ (and $^{13}C/^{12}C$) are not necessarily evident at the molecular level and thus only part of the physiological and/or climate related signal can be extracted. Instead it is more evident at a site-specific level (Gleixner and Schmidt, 1997; Schmidt et al., 2003).

To realize such potential, the first step is to develop an analytical method that can access the site-specific $^{2}H/^{1}H$ ratios within a single carbohydrate molecule. Taking advantage of an NMR-based technique (^{2}H-SNIF-NMR: site-specific natural hydrogen isotopic fractionation studied by nuclear magnetic resonance) pioneered at the Université de Nantes in the early 1980s (Martin and Martin, 1981), Zhang et al. (1994) first obtained data showing that intramolecular ^{2}H profiles of C_3 and C_4 (endosperm starch) glucose are different. Later work by the same group, as well as from Schleucher and

* Corresponding author. School of Chemistry & Chemical Engineering, Shaanxi University of Science & Technology, Xi'an, China.
E-mail address: youping.zhou@sust.edu.cn (Y. Zhou).

https://doi.org/10.1016/j.phytochem.2017.11.004
0031-9422/

coworkers at Umeå University, confirmed such C_3-C_4 differences exist also in soluble sugars (including fructose), leaf starch and cellulose (Schleucher, 1998; Schleucher et al., 1998, 1999; Zhang et al., 2002; Betson et al., 2006; Augusti et al., 2006, 2007). From the illuminating work of these two groups, it was clear that ***i***) the intramolecular distributions of ^2H are non-random for both C_3 and C_4 plant glucoses; ***ii***) the H^1 and H^6 (the average of the pro-chiral Hs, and in particular $H^{6,\ pro\text{-}R}$) of C_4 glucose are always enriched, while H^4 and H^5 are always depleted in ^2H relative to the average of all 7 C-Hs (see Fig. 1 for glucose structure and the numbering of C-H atoms). In C_3 plant glucose, the profile is the opposite: H^1 & H^6 (and in particular $H^{6,\ pro\text{-}R}$) are always depleted, while H^4 & H^5 are always enriched in ^2H relative to the average of all 7 C-Hs. Notwithstanding these revealing intramolecular measurements, little effort has been made so far to explain what is causing such intramolecular ^2H profile differences between C_3 and C_4 glucoses. Valuable metabolic and photosynthetic information hidden in those profiles is thus not released but would be crucial for the full exploitation of physiological and environmental signals in natural archives.

In a recent effort made by Zhou et al. (2016) to provide a biochemical explanation for the molecular level C_3 *versus* C_4 ^2H differences of C-H in lipids synthesized via three independent pathways (fatty lipids via the acetogenic (ACT) pathway, phytol via the 1-deoxy-D-xylulose 5-phosphate (DXP) pathway and sterols via the mevalonic acid (MVA) pathway), it was suggested that pyruvate, the common precursor (for the three pathways) and NADPH used for lipid biosynthesis in C_4 plants have different isotopic compositions from those in C_3 plants due to photorespiration in C_3 and compartmentation of water (the primary hydrogen source) in C_4 plants. As lipid biosynthesis and carbohydrate metabolism in leaves are interrelated by triose phosphates, it is reasonable to assume that the biochemical mechanisms behind the molecular level differences between C_3 and C_4 lipids may also be evident in the intramolecular (positional level) ^2H distribution difference between C_3 and C_4 carbohydrates.

Providing a reasonable (and quantitative) biochemical explanation for the intramolecular ^2H profile of carbohydrate is a big challenge as there are many steps where H isotope effects can occur due to bond cleavage and formation, isotope exchange between and among metabolic intermediates and solvents (H_2O) and metabolic branching (Schmidt et al., 2003). In this VIEWPOINT, we have made the first attempt to rationalise the contrasting intramolecular profiles and by laying out a tentative framework, we hope to encourage others to fill in gaps or modify areas of ambiguity.

We propose that photorespiration and compartmentation contribute to the observed C_3 *versus* C_4 differences in glucose intramolecular ^2H profiles. We specifically describe 1) the likely mechanisms behind the relative enrichment of H^4 and H^5 in C_3 plant and relative depletion in C_4 plants, 2) the isotopic differences between C_3 and C_4 glucoses in H^1 and H^6, and lastly, 3) discuss the ^2H distribution in C_3 glucose as affected by growth.

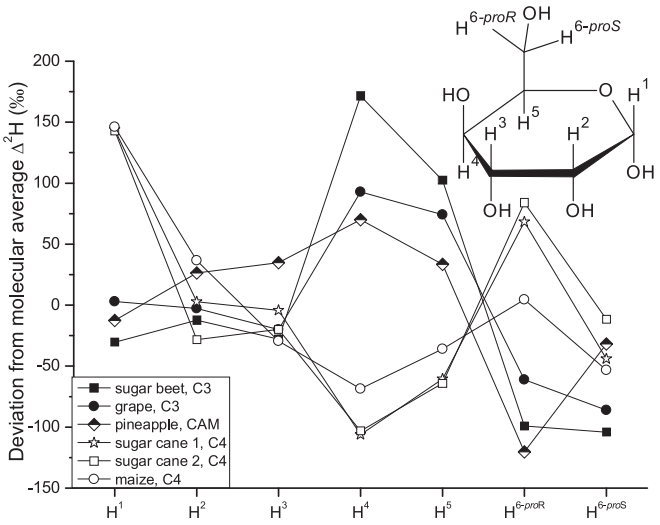

Fig. 1. Intramolecular ^2H distribution of glucose in fruit of C_3 (sugar beet and grape) and CAM (pineapple), and C_4 (sugarcane and maize, both NADP-ME) plants using the data of Zhang et al. (2002). The original $^2H/^1H$ data (in ppm) were converted to δ^2H using δ^2H (‰) = $[[(^2H/^1H)_{sample}-(^2H/^1H)_{SMOW}]/(^2H/^1H)_{SMOW}]$*1000, where $(^2H/^1H)_{SMOW}$ is 156 ppm and are expressed as deviation (Δ^2H) from the average of all 7 C-Hs (carbon-bound hydrogen atoms) in glucose. Similar profiles for leaf sugars, starch and stem carbohydrates were also reported by Schleucher (1998), Schleucher et al. (1999), Augusti et al. (2006), Betson et al. (2006), Augusti (2007, PhD thesis), and Augusti et al. (2008).

2. Relative ^2H enrichment at H^4 & H^5 in C_3 glucose

Since there is no reason to believe that the biochemistry of C_4 CBB (Calvin-Benson-Bassham) cycle leading to the synthesis of glucose is different from that in a C_3 cell, it is reasonable to assume that there are no step-specific differences in the isotope effects associated with the biochemical reactions between a C_3 cell and a C_4 cell (regardless of the cell types). From this assumption, it follows that position-specific isotopic differences between C_3 and C_4 glucose will be determined by the isotopic compositions of cellular water in which the biochemistries occur, the NADPH involved in reduction and the isotopic effects associated with their incorporation into and "shuttling" among the positions to which the H atoms (in question) are covalently attached.

Enrichment of H^4: when the two PGA molecules (**2a** and **2b**, the former is usually referred to as the "upper" PGA and the latter the "lower" PGA in most of the RubisCO biochemistry) generated at the first (carboxylation) step of the CBB cycle (Fig. 2) is converted to GAP (**4a**) under the catalysis of GAPDH, a H^- from the chloroplastic NADPH is introduced into the H^1 of GAP (**4a**). As the NADPH -derived H is known to be highly depleted in ^2H (Luo et al., 1991) and that the donation of an H^- is predicted to have a large kinetic isotope effect KIE (Miller and Hinck, 2001), the H^1 of GAP (**4a**) (H^4 of glucose) should therefore be even more depleted relative to the chloroplastic NADPH transferable hydrogen. This is, however, contrary to the observation that H^4 is most enriched in C_3 glucose (Fig. 1 and **8a**, **8b**, **9a**, **9b,** starch and sucrose in Fig. 2). We provide below two mechanisms to account for the apparent enrichment at H^4: ***i***) during the conversion of H^2 of GAP (**4a**) to $H^{1,\ pro\text{-}R}$ of DHAP (**5a**), H of cellular (chloroplastic) water (H_2O) is also introduced (in the form of a proton H^+) into DHAP (**5a**) and presumably also into H^1 of GAP (**4a**) (O'Donoghue et al., 2005a,b; Pionnier and Zhang, 2002; Pionnier et al., 2003, Fig. 3), the isotopically relatively enriched cellular water passes on the enrichment to H^1 of GAP (**4a**) and H^4 of glucose (**9a**); ***ii***) as suggested by Tcherkez (2010), an EIE (equilibrium isotope effect) of TPI which enriches H^1 of DHAP (**5a**), may also contribute to the enrichment at H^1 of GAP (**4a**) (H^4 of glucose). Of the two possible mechanisms, we rank the first one more likely as it not only accounts for the H^4 enrichment in C_3 glucose but also explains in part the H^4 depletion in C_4 glucose (see the next section). The ^2H-enriched cellular (chloroplastic) water alone, or in combination with the EIE-caused enrichment, more than compensates for the depletion due to the introduction of ^2H-depleted (transferable H of) NADPH to H^1 of GAP (**4a**).

We also argue that the enrichment of H^5, which comes from H^2 of GAP (**4a**) is a result of photorespiration in C_3 cells. First, the transferable hydrogen in NADH in the peroxisome is enriched in ^2H due to transhydrogenation to form NADPH (see Fig. 2-the