

Review

Molecular hydrogen isotope analysis of living and fossil plants—*Metasequoia* as an example

Hong Yang^{a,b,*}, Qin Leng^{a,c}

^a Department of Science and Technology, College of Arts and Sciences, Bryant University, Smithfield, RI 02917, USA

^b Department of Geology and Geophysics, Yale University, New Haven, CT 06520, USA

^c LPS, Nanjing Institute of Geology and Palaeontology, CAS, Nanjing 210008, China

Received 25 August 2008; received in revised form 15 September 2008; accepted 19 September 2008

Abstract

Molecular hydrogen isotope analysis performed on modern and fossil plants has made a significant impact on diverse research fields in biology and geology. Using living and fossil *Metasequoia* as an example, we review the technology of online GC-IRMS that made the molecular analysis of hydrogen isotope possible and discuss critical issues concerning with the studies of molecular δD and its applications. The apparent hydrogen fractionation factors between lipid molecules and source water ($\epsilon_{\text{water-lipid}}$) vary across plant taxonomy and differ among biomolecules and are affected by multiple environmental factors in which precipitation δD values exercise the first order of control. Eco-physiological factors and environmental parameters are also known to influence δD in plants. Molecular hydrogen isotope analysis of chemically stable lipid molecules, such as *n*-alkanes, finds a wide range of applications in detecting source sediments, reconstruction of paleoclimatic parameters, inference of air-mass trajectory, as well as in petroleum industry and environmental studies. © 2009 National Natural Science Foundation of China and Chinese Academy of Sciences. Published by Elsevier Limited and Science in China Press. All rights reserved.

Keywords: Compound-specific δD ; Plant molecule; Hydrogen fractionation; Paleoclimate; *Metasequoia*

1. Introduction

Hydrogen is one of the most abundant elements on the Earth, and is fundamental to the constitution of organic molecules that are essential to all life forms. Hydrogen has two stable isotopes: protium (^1H , or H) and deuterium (^2H , or D). Casually, “hydrogen” is referred to the former whereas the latter is called “heavy hydrogen.” Ever since Urey’s Nobel Prize-winning discovery of heavy hydrogen [1], research on hydrogen isotope has advanced our knowledge in many aspects of biology and geology. Green plants acquire their hydrogen atoms through photosynthesis,

incorporating hydrogen from the environmental water that is largely controlled by precipitation. Due to the large isotope effects of hydrogen and the large deuterium/hydrogen (D/H) variation known to exist in nature, hydrogen isotope has been an attractive candidate for biological and geological applications. However, the small quantity of naturally occurred deuterium (0.015% of hydrogen) has traditionally posted an analytical challenge for an accurate measurement of D compositions in geological and biological samples, thus the studies of stable hydrogen isotopes in modern and fossil plants have stayed behind those of molecular carbon isotopes [2]. Only recently, technological breakthroughs on isotope ratio monitoring gas chromatography/thermal conversion/mass spectrometry (irm-GC/TC/MS, or IRMS) [3–5] made it possible to precisely determine hydrogen isotopes at the molecular level in modern and fossil plants, also known as compound-specific hydro-

* Corresponding author. Tel.: +1 401 232 6223; fax: +1 401 232 6068.
E-mail address: hyang@bryant.edu (H. Yang).

gen isotope analysis. Up-to-date results have demonstrated the potential and the feasibility of applying this technology to paleoenvironmental (including paleoclimatological) analysis [6–10], making molecular hydrogen isotope analysis a new frontier in geobiological research.

Due to the known source and the stability of lipid molecules, compound-specific hydrogen isotope analysis so far has been focused on lipids with various carbon chain lengths, such as C_{25} – C_{31} *n*-alkanes which are believed to have derived exclusively from leaf waxes of higher plants. The advantage of using ancient biomolecules from known fossil material (*in situ*) takes account of taxon-specific hydrogen isotope fractionations that have been shown as an important factor in determining D/H variations among plants [7,11]. However, taxon-specific analysis on molecular hydrogen isotope from fossil material has been rare, and the full potential in molecular paleobotanical studies has not been fully exploited. Integrated with traditional paleobotanical approaches and isotope analysis of other elements, such as carbon and nitrogen, compound-specific hydrogen analysis has become a powerful tool for both biologists and geologists.

With the rapid accumulation of global lipid δD data from higher plant waxes, in both living and fossil material, it becomes possible to summarize hydrogen isotope compositions of leaf waxes in relation to various biological and environmental factors. Here, we review the current knowledge, key issues, and applications of molecular hydrogen isotope analysis on both living and fossil plants. We attempt to achieve the following goals: (1) illustrate the new technology of online GC-IRMS that made the compound-specific analysis of hydrogen isotope possible; (2) discuss the incorporation of hydrogen into plant tissues and its associated fractionations at the molecular level; (3) explain the observed variability of δD in plant lipids in relation to various environmental factors; (4) summarize our current understanding on preservation of compound-specific δD signals in the geological record; and (5) outline the potential for using plant molecular D/H data for ecological and paleoecological studies.

The deciduous conifer *Metasequoia* Miki 1941 (Cupressaceae) is perhaps one of the very few higher plants that have received molecular hydrogen studies on both living and fossil material. Exceptionally preserved fossil *Metasequoia* from the Arctic Tertiary deposits [12,13] provides opportunities to determine hydrogen isotope compositions of individual lipid molecules. We use molecular hydrogen isotope data recently obtained from this model plant to demonstrate the potential of unlocking ecological and paleoenvironmental information. To our knowledge, this paper represents the first attempt to review the current knowledge in the merging field of molecular hydrogen analysis of plants. We provide a comprehensive and up-to-date list of references so far published in this field. As the compound-specific hydrogen analysis has a broad application, those in the petroleum field [14–21], environmental studies [22–28], and carbonaceous meteorite research [29–32] are

beyond the scope of this paper; interested readers can refer to the included references for recent progresses.

2. Molecular hydrogen isotope technology

The key technology to analyze molecular hydrogen isotope ratios from living and fossil plants has to meet the following challenges: (1) extract, isolate, and concentrate biomolecules that may contain only low quantity of hydrogen and deuterium; (2) quantitative conversion of organic molecular hydrogen to hydrogen gas (H_2) to be measured precisely by mass spectrometer; (3) correction of H_3 factors. One of the major difficulties for measuring hydrogen isotope composition is its small quantity. Compared with ^{13}C enrichment in natural samples (1.1% of ^{12}C), deuterium has a mean natural abundance of about 35 times smaller. Given the fact that hydrogen gas is more difficult to be ionized than carbon dioxide, the analytical system of obtaining δD values requires 700 times more sensitive than that used for carbon isotopes [5]. Consequently, up to 20 times more samples and a more effective extraction and separation technique are required for molecular hydrogen isotope analysis in comparison with that of carbon isotopes. Thus, it poses a challenge for recovering *in situ* lipids from identifiable fossils. For example, 1.5–2 g of well preserved fossil *Metasequoia* samples from the Canadian Arctic Early Paleogene deposits were used to prepare 200–300 ng *n*-alkane molecules for a triplet D/H analysis as required.

Critical steps to measure individual molecules from small quantity but mixed organic matters include extraction, separation, and purification of desired molecules before samples can be measured using an isotope ratio mass spectrometer (Fig. 1). Extraction of modern plant lipids can be achieved simply by using organic solvent (e.g., DCM) to wash leaf surface covered by waxes. Traditional organic extraction employs techniques such as the soxhlet method or the application of accelerated solvent extractor (ASE) that extracts total lipid under high temperature (100 °C) and pressure (100 bar) using 2:1(v/v) dichloromethane(DCM):methanol for fossils or sediments [9]. Solid phase ion exchange columns are used to fractionate extracted total lipids into different fractions by silica gel

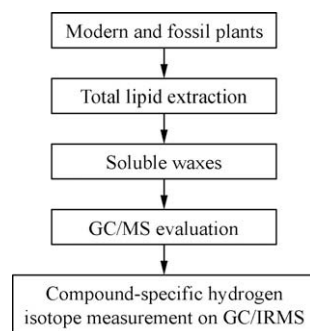


Fig. 1. Experimental flowchart for obtaining molecular hydrogen isotope composition from modern and fossil plants.

flash column chromatography using solvents of increasing polarity. The purity and abundance of extracted lipid molecules can be examined using a gas-chromatography (GC).

The measurement of D/H on individual lipid molecules involves continuous-flow mass spectrometry in which the continuous conversion of organic hydrogen to hydrogen gas in a stream of carrier gas, usually helium, allowing a precise measurement of D/H ratios for each H₂ peak [5]. The cornerstone of the continuous-flow IRMS technique is to quantitatively convert hydrogen bound in organic compounds into hydrogen gas prior to analysis in IRMS at the temperature of 1400 °C. The pyrolysis process is known as thermal conversion [3]. GC separated compounds pass through the heated alumina tube to convert organic H to H₂ which is then introduced into an isotope ratio mass spectrometer (usually a Delta^{plus}XL or a Finnigan 253), which determines D/H ratios of individual compounds by integrating beams of different mass signals [5] (also illustrated in Fig. 2). Three standards (*n*-C₁₆ and *n*-C₃₀ alkanes, 5 α -androstanes) with known D/H ratios determined off-line are commonly co-injected to monitor the analytical accuracy.

The continuous-flow IRMS system has several advantages. First, the connection between GC and a combustion device allows the analysis of samples with mixed organic compounds. Separation and selection of analyzed compounds were achieved prior to IRMS analysis. This is ideal for analyzing geological samples that usually contain a mixture of natural compounds. Second, recent development and improvement of the technique have provided sensitivities

required for hydrogen isotope measurement from a variety of geo-lipids which can be recovered only in relatively small quantity. Third, measurement of D/H ratios of individual compounds can now be made to a precision of 2‰ or better. Finally, as the prepared sample can be used for both online isotope analyses for carbon and hydrogen isotopes, multiple isotope measurements can be carried out on the same biomolecules through one sample preparation. Thus, the ability of measuring individual compounds opens up new research areas to study plant ecology and paleoecology.

In the IRMS ion source, H₃⁺ is formed through the reaction between H₂⁺ and H₂, causing an overestimation of 3(HD):2(H₂) ratio. Thus, correction of H₃⁺ contribution in IRMS becomes an important issue in minimizing errors related to the H₃ factor [33]. In practice, the H₃⁺ correction factor (*k*) is reviewed on a daily basis with a known standard (hexane for example) on the IRMS. The standard delta notation (‰ Vienna standard mean ocean water, or VSMOW) for reporting hydrogen isotope values (δD) is followed in $\delta D = 1000 \times [(^2H/^1H)_{\text{sample}} / (^2H/^1H)_{\text{VSMOW}} - 1]$, and in apparent hydrogen isotopic fractionation factors ($\epsilon_{\text{water-lipid}}$) in $\epsilon_{\text{water-lipid}} = 1000 \times [(\delta D_{\text{water}} + 1000) / (\delta D_{\text{lipid}} + 1000) - 1]$ [34].

3. Hydrogen incorporation into plant tissues and associated isotopic fractionations

Classic works on hydrogen isotope biochemistry of modern plants have laid out the foundation for the

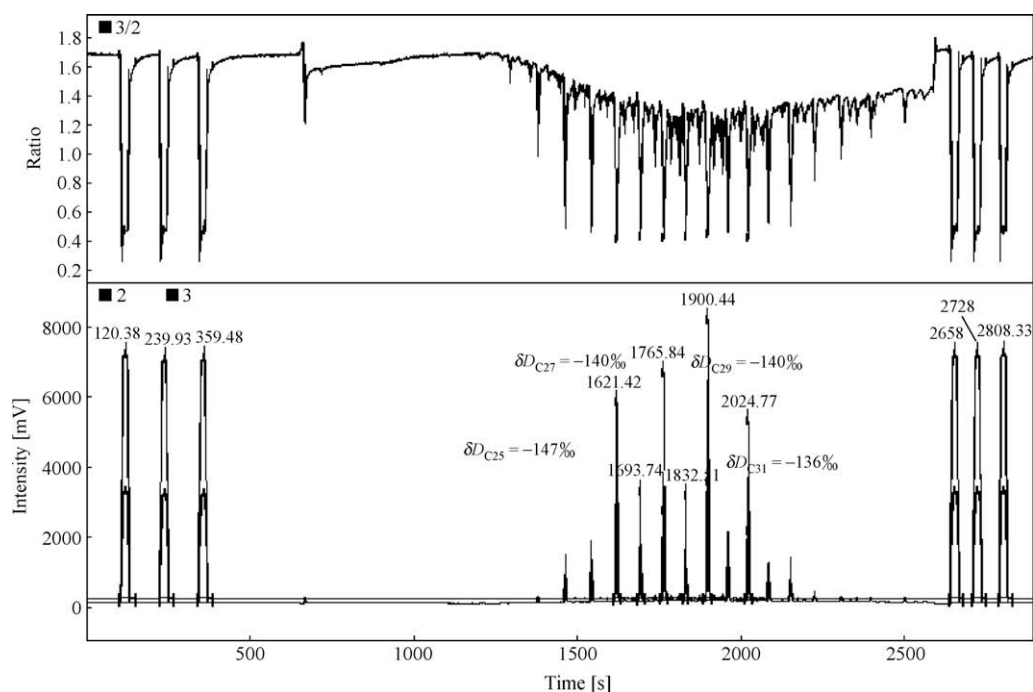


Fig. 2. GC-IRMS (Finnigan 253) generated H₂ signal of 1 µl injection of *n*-alkanes isolated from leaves of living *Metasequoia*, showing mass 3(DH)/2(H₂) ratios (upper trace) and the intensity of mass 2(H₂) along with time (second). The first and last three peaks are reference gases, and δD is labeled along with identified major peaks.

understanding of how hydrogen is fixed into green plants and how subsequent hydrogen fractionations may lead to hydrogen isotope compositions in different plant tissues (reviewed in Refs. [35,36] (also see Fig. 3 for illustration). Smith and Epstein [37] observed D-depleted plant tissues relative to environmental water and pointed out that various metabolic processes have resulted in different degrees of fractionation for hydrogen in different plant tissues. Schiegl and Vogel [38] detected that fossil fuels (i.e., coal and oil) are further depleted in D with respect to living plants. With the development of the isotope technology to study non-exchangeable hydrogen in plant cellulose [39,40], a wide variation of δD values across different plant taxa and among different tissues within a single plant has become evident. Studies on cellulose and plant bulk organic matter have indicated that D/H ratios of organic matter in terrestrial plants are primarily influenced by source water rather than metabolic or physiological process [40,41]. Furthermore, it is believed that water is not altered isotopically during uptake by roots, except for woody xerophytes in which small amounts (3–9‰) of hydrogen isotope fractionation have been observed [42]. Hydrogen isotope compositions in plant leaf tissues are further affected by two major fractionations in opposite directions [43,44]: evapo-transpiration resulting in an enrichment in D in the leaf water [45,46] whereas lipid biosynthesis processes leading to fractionations against D [37,43,47].

To understand the mechanism and magnitudes of apparent hydrogen fractionation between a particular lipid molecule and medium water ($\epsilon_{\text{water-lipid}}$) is the key to apply plant D/H values for paleoenvironmental studies. From the very beginning of molecular investigations of hydrogen isotope in living plants [8], it has been clear that different biosynthetic fractionations in plants lead to a wide array of δD values in different plant biomolecules [48,49]. Among different classes of biochemicals in plants, lipid molecules are more depleted in D relative to bulk samples [50]; and even among different lipid molecules, the offset between straight-chain lipids and phytol lipids can be more than 200‰ [8]. Chikaraishi and Naraoka [51] reported similar apparent hydrogen fractionation factors in *n*-alkanes from C_3 angiosperms and gymnosperms; Sachse et al. [52] further stated that biochemical fractionations for *n*-alkanes are less influenced by environmental factors, suggesting that biochemical fractionations in plants might be constant (for example a mean value of -128‰ for C_3 plants). However, recent work indicated that $\epsilon_{\text{water-lipid}}$ not only varies considerably among plants with different taxonomies [11] or life forms [53], but also is impacted by climate gradient [54] or even light irradiation (Yang et al. unpublished data). In addition, biosynthetic fractionations experience temporal changes in different seasons for a single plant using source water of constant hydrogen isotopes [55]. Usually, hydrogen isotopic fractionations in biochemical systems are large, thus making calculation involving two

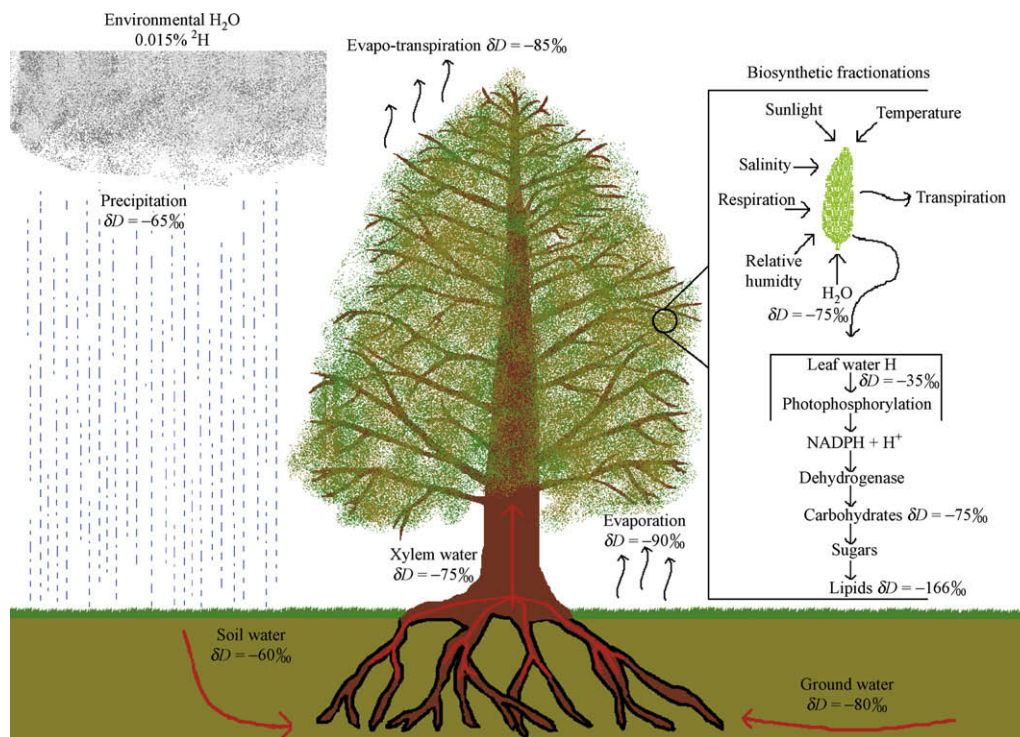


Fig. 3. A quantitative model for hydrogen isotopic composition of plants in relation to the environmental water. Various δD values were based upon the experimental data collected from a tree of *Metasequoia glyptostroboides*, a C_3 conifer in the family Cupressaceae, living at 45°N in the USA with a $\epsilon_{\text{water-lipid}}$ of -105‰ (Yang et al. unpublished data). Intermediate δD values were estimated based upon fractionations previously reported in other plants [36,37,43,82,112–114].

or more hydrogen sources a challenging task [56]. Nonetheless, the application of taxon-specific and compound-specific apparent hydrogen fractionations would provide a more reliable means to reconstruct ancient D/H ratios in source water [7].

In addition to higher plants, lipid δD from algae also exhibits strong correlation with water D/H ratios [57]. Values of hydrogen fractionations between lipids and water are significantly smaller in bacteria (for example *Methylococcus capsulatus*) than those in photosynthetic organisms such as cyanobacteria [58], algae [59,60], and higher plants [61]. Even while H_2 is present under anaerobic conditions, D/H ratios of fatty acids in bacteria are in isotopic equilibrium with water medium [62,63]. A recent study by Jones et al. [64] indicates that dissolution or degradation of organic compounds may also influence hydrogen fractionations of marine microbes. Furthermore, studies on compound-specific hydrogen fractionations were also carried out for organic compounds that go through progressive vaporization [65] or UV degradation [66].

4. Factors controlling D/H variations in higher plants

Since green plants acquire hydrogen solely from environmental water, D/H ratios in plant tissues should correlate well with D/H ratios from precipitation. However, in natural conditions, the relationship is not straightforward. Lawrence and White [67] reported a strong correlation between D/H ratio of summer precipitation and tree-ring cellulose from *Pinus strobus* L. in New York and the amount of rainfall between May and August. Roden and Ehleringer [68] and Roden et al. [69] showed that in addition to water source, humidity information was also recorded in tree-ring cellulose. Recent work at the molecular level based upon global *n*-alkane data clearly indicates a multiple control of hydrogen isotope composition and its variability in plant leaf waxes [6]. These different levels of controls imposed different magnitudes of variation on plant δD . Among them, precipitation δD values, controlled by the global latitudinal gradient, act as the dominating factor that exercises the first order of control for hydrogen isotopic compositions of plant leaf wax at the global scale [6].

It has been observed that there is a considerable amount of taxonomic variations of D/H ratios for a given plant molecule, even from plants that utilize the same source water [7,11,57]. Fig. 4(c) shows different D/H ratios from *n*-alkanes of *Metasequoia glyptostroboides* and *Glyptostrobus pensilis* Koch, two closely related conifers that grow under the same greenhouse environmental conditions with the same source water. Obviously, factors in addition to source water are involved. Liu et al. [7] have revealed that the ecological life forms of higher plants play an important role in controlling hydrogen isotopic compositions of leaf wax *n*-alkanes by tapping into different water pools and possibly by means of different mechanisms of biological fractionations. They found out that while grasses and woody plants grow side by side, the grasses usually possess

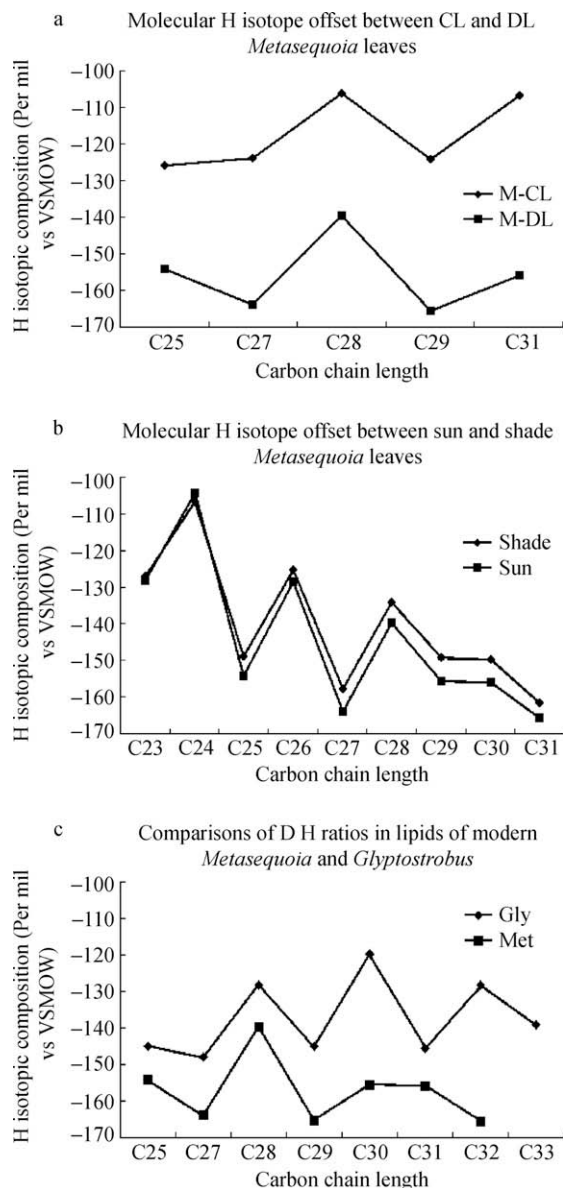


Fig. 4. Molecular hydrogen isotope values from modern *Metasequoia* and *Glyptostrobus*. (a) Impact of different light irradiation. CL, continuous light; DL, diurnal light (at 45°N). (b) Little change between sun and shade leaves. (c) Taxon-specific molecular hydrogen isotope signatures between two closely related genera. Gly, *Glyptostrobus*; Met, *Metasequoia*.

more negative D/H ratio than co-occurring trees and shrubs. On the other hand, trees grow next to a stream use little or none of the surface stream water as their roots may penetrate the ground water aquifer [70]. In addition, on a global scale, woody plants and grasses have different responses to the changes of global precipitation δD , pointing toward the importance of plant life forms in affecting D/H ratios in leaf waxes [6].

Physiological features, including photosynthesis pathways [8,11,50], water-use efficiency [11], evapo-transpiration [71], and light irritation (Yang et al. unpublished data), can also leave an imprint on δD patterns of plant

leaf waxes, causing δD variations. Early work at the tissue level has indicated that D/H ratios in tissue water may be used to discriminate higher plants with different biosynthetic pathways (C_3 , C_4 , and CAM plants) [72]. The fact that $\delta^{18}O$ and δD behave differently in certain higher plants lead to the belief that biological fractionations, presumably due to biosynthetic pathways and physiological differences during hydrogen incorporation, appeared to be as important as environmental factors [73,74]. Cross-plotting with molecular C isotope fractionation data, Chikaraishi et al. [50] were able to discriminate lipids produced by different biosynthesis pathways: acetogenic, mevalonic-acid (MVA), and 2-C-methyl-D-erythritol-4-phosphate (MEP). Similar control of hydrogen fractionations by alternative biosynthetic pathways can also be observed by examining chlorophyll biosynthesis in C_3 plants [75]. Furthermore, Smith and Freeman [71] observed 20‰ D enrichment in n -alkanes of C_4 plants compared with co-occurring C_3 plants, and they attributed the difference to the degree of transpiration due to different plant physiologies. However, Bi et al. [76] have reported that δD from n -alkanes is not a diagnostic discriminator for C_3 and C_4 plants, and Liu et al. [7] found no significant difference between average δD values of n -alkanes in C_3 and C_4 plants from the Chinese Loess Plateau. By comparing δD and $\delta^{13}C$ of n -acids from 35 higher plants, Hou et al. [11] suggested that plant water-use efficiency also controls δD values of leaf waxes in trees. In addition, greenhouse experiment using controlled light conditions demonstrates that continuous light under simulated Arctic summer conditions impacts D/H compositions of leaf n -alkanes in deciduous conifers. Plant n -alkanes from conifer leaves treated with 4 months of continuous light display more positive D/H values than those from their counterparts under normal light at 45°N (Fig. 4(a)). Unlike the isotopic behaviors of carbon isotope, δD values of n -alkanes from sun leaves and shade leaves of a single tree bear no statistical difference as shown in *Metasequoia* (Fig. 4(b)). Recent experimental data obtained from plants in growth chambers indicated that hydrogen isotope ratio of plant leaf waxes are not affected by at least 2-fold of pCO_2 change [77]. On one occasion, hydrogen flow in a food chain was demonstrated to have left a footprint on δD values. Chikaraishi [78] investigated hydrogen isotopes of sterols from brown and red algae and shellfish feeding on these algae. His finding indicates that both $\delta^{13}C$ and δD values of sterols (except for cholesterol) from shellfish are similar to those in brown and green algae, implying a dietary relationship and suggesting no significant hydrogen fractionation occurred during the up-taking of algae by shellfish.

The biological and geological significance of δD variation in plant biomolecules is dependent upon a better understanding of the controlling environmental and biological factors, and consequently a better interpretation of δD data from compound-specific analysis of hydrogen isotope compositions from sediments and geological samples. Available evidence suggests that knowledge of the fac-

tors affecting responses of individual plant taxon to environment is necessary to accurately infer paleoclimate from D/H ratios of molecular hydrogen isotope [6,7,11,57,71].

5. Preservation of molecular hydrogen signals in the geological record

Preservation of D/H ratios in fossils and ancient sediments bears tremendous interests to geologists and geochemists because it has direct impacts on the interpretation of hydrogen isotope signals in the geological record [9,79–81]. Preservation of hydrogen isotope signals concerns with two broad issues: (1) Isotopic exchange of organic hydrogen with environmental water and (2) diagenetic alteration of preserved D/H ratios. The bonding strength between H and other atoms, C for example, largely controls the rate of hydrogen exchange between organic molecules and available environmental media (usually water), although the functional group, structure of the molecule, and stereochemistry also play a role [80,81]. Early studies have indicated that carbon bound hydrogen atoms do not exchange with water in natural condition [82]. A number of recent publications have dealt with the change of D/H ratio during sediment maturity [14,17,18]. Both experimental data and down-core observations have indicated that n -alkyl is the most resistant to exchange, while isoprenoids (pristine and phytoane) are the most prone to hydrogen exchange [17,18]. Chikaraishi [78] has found out that δD values of sterols isolated *in situ* from brown and green algae are consistent with those of algal sterols in marine sediments, indicating the preservation of isotope signals of sterols in the sedimentary matrix. Yang and Huang [9] were the first to have demonstrated the preservation of endogenous D/H values in individual lipids of n -alkanes and n -acids from plant fossils and sediments of the exceptionally preserved Clarkia Miocene deposit in northern Idaho, USA, suggesting minimal hydrogen isotope exchange after deposition in immature sedimentary depositional system. Studies on D/H ratios in *Metasequoia* n -alkanes through a decay series as well as in exceptionally preserved Eocene fossils have clearly established that ancient D/H signals can be recovered (Yang et al. unpublished data). The similarity of D/H ratios through a decay series (variations within technical errors) and the regular offset among different compounds suggest minimal hydrogen exchange in these samples. It is believed that chemically stable lipid molecules, such as n -alkanes, as old as in Cenozoic-aged (if not older) immature sediments, preserve original molecular hydrogen isotope signals. While the preservation of D/H values in older and/or mature sediments waits for further evaluation, the negligible effect on the primary deuterium concentrations of these resistant lipid molecules in Cenozoic deposits warrants various geological applications,

thus opening the door to a series of geobiological studies.

6. Applications of molecular hydrogen isotope data

6.1. Source of sediments

As organic matters in natural settings are mixed from multiple aquatic and terrestrial sources, the ability to distinguish contributing sources of these organic material is a critical step towards various geological applications. In a number of occasions, D/H ratios in sediments have been used, in conjunction with $\delta^{13}\text{C}$ data or with alkenone unsaturation ratios U_{37}^k , to determine specific source of contributions of biomarkers [50,60]. Using compound-specific $\delta^{13}\text{C}$ – δD data, Chikaraishi and Naraoka [83] were able to evaluate the source input for phytol, sterols, and *n*-fatty acids in Japanese lacustrine deposits. Applying the same approach, Chikaraishi et al. [49] stated that chlorophyllide α in sediments at the Lake Haruna in Japan were mostly derived from phytoplanktons, whereas phytol and sterols were contributed from terrestrial sources. To reconcile alkenone unsaturation ratios with their δD values from Sargasso Sea, Englebrecht and Sachs [84] invoked a three sourced model to explain the depleted δD values obtained from alkenones in late Holocene sediments from the Bermuda Rise.

6.2. D/H ratios of ancient lake water

Because plant hydrogen isotope has a direct link to the atmosphere (via moisture) and hydrosphere (via precipitation), compound-specific hydrogen isotope analysis holds great promises for quantitative paleoclimatological reconstructions. As plant δD data are expected to carry hydrogen isotope information of environmental water, considerable efforts have been made to establish a quantitative correlation between plant δD and source water δD . The organic isotope approach is particularly attractive to the researchers working on lacustrine deposits in which suitable carbonate materials in sediments are usually lacking. It is known that δD of total lipids from submerged aquatic plants records δD of environmental water [47]. At the molecular level, Sauer et al. [85] demonstrated that δD of aquatic algal sterols isolated from lake sediments have a linear relationship with δD of lake water, thus these sterols in freshwater systems can serve as a useful biomarker to reconstruct D/H ratios in lake water. Huang et al. [86,87] argued that hydrogen isotope compositions of palmitic acid (C_{16} *n*-acid) ($\delta\text{D}_{\text{PA}}$) capture the δD signals of lake water. Based upon an empirically established correlation between $\delta\text{D}_{\text{PA}}$ and lake water δD and in collaboration with the data from pollen and other proxies, they have inferred Quaternary climatic trends in New England [88,89]. However, recent experimental data on hydrogen isotope fractionation in cultured freshwater algae have shown that $\delta\text{D}_{\text{PA}}$ can vary significantly (up to 100‰)

across different algal species [57]. Thus, cautions are expressed in applying empirically derived water–lipid apparent fractionation factors between lake water δD and $\delta\text{D}_{\text{PA}}$ for paleoclimatic reconstruction without the knowledge of source algal species.

6.3. δD and temperature

Early studies on tree cellulose from a wide geographic range over North America have observed a distinct relationship between δD of carbon bound hydrogen in cellulose and average annual temperature [90]. Further research found out that such a correlation is particularly good for low frequency variations over long-term trends [91]. Variations may occur due to other hydrological and biological factors such as humidity, topography, and different species [92]. One of the earliest case studies using molecular hydrogen isotopic values from *Sphagnum* bog found a high statistical correlation between δD in *n*- C_{23} (*n*-tricosane) and recorded temperature for the past 200 years in Cumbria, UK [93]. Interestingly, the correlation was enhanced under the following two conditions: (1) *In situ n*-tricosane from modern *Sphagnum* was used and (2) summer temperature data were applied. These results implied that the background noise may not be due to temporal variations in *Sphagnum* population but rather caused by changes of temperature related climate parameters. Working on sedimentary samples from five lakes covering different climates and different tropic states in Europe, Sachse et al. [52] reported that algal derived *n*- C_{17} δD values are positively correlated with mean annual temperature, whereas the correlation is negative for *n*- C_{16} and *n*- C_{18} : An interesting result that may suggest different sources of compounds from different organisms. Using multiple proxies, Hu et al. [94] believe that the observed 20‰ increase in $\delta\text{D}_{\text{PA}}$ from Alaska sediments reflects an abrupt temperature increase between 16,000 to 13,300 years before present. The correlation between D/H ratios in plant leaf waxes and atmospheric temperature seems to be co-variant with other climate parameters (such as humidity), and the intricate relationship between temperature and precipitation makes the task of inferring temperatures even more challenging. Nevertheless, reconstruction of temperature signals from molecular hydrogen isotopic compositions in plants will undoubtedly attract more research in the near future.

6.4. δD and salinity

In addition to the application of alkenones as a paleotemperature proxy, D/H ratios from marine alkenones were found to closely track D/H ratios of environmental water [84]. Recent work on hydrogen isotope compositions from aquatic algae has led to the postulation that water salinity may play a critical role in controlling D/H values of individual lipids from aquatic algae. Recent technical improvement of IRMS has circumvented previous problems of

chromatographic coelution of alkenones with different numbers of double bonds, allowing accurate measurement of D/H ratios of individual alkenones [95]. Working on hydrogen isotope of long-chain alkenones from algae (*Emiliania huxleyi* and *Gephyrocapsa oceanica*) under controlled experimental setting, Schouten et al. [96] noted that the hydrogen fractionation factor between alkenone and water was correlated with water salinity and growth rate. The substantial impact of hydrogen isotope fractionation due to salinity and growth rate is explained by the differential D/H values between water inside and outside of cells derived from metabolic process during synthesis of alkenones at different growth rates. To apply these correlations to field studies, van der Meer et al. [97,98] examined D/H values of C₃₇ alkenones in sediments from the Mediterranean Sea and the Black Sea and suggested that while the growth rate is constrained, D/H values of long-chain alkenones can be used to estimate the changes of sea surface salinity (SSS). Sachse and Sachs [99] recently expanded these studies by investigating D/H values of cyanobacterial lipids from hypersaline lakes. They have observed a strong positive correlation between D/H values of a suite of lipids and water salinity with the absence of substantial increase in lake water δD . However, their study points out that the dependency of D/H fractionation in cyanobacterial lipids on salinity perhaps has occurred during water transporting into cells prior to lipid biosynthesis. Nonetheless, the salinity dependency of lipid D/H fractionation provides a challenge for using D/H ratios derived from lipids of aquatic microorganisms to a temperature proxy while offers an opportunity to reconstruct paleosalinity in ancient environments using taxon-specific and compound-specific hydrogen isotope analysis.

6.5. Paleo-hydrological changes

Once the relationship between δD of individual lipid compounds and δD of source water is established, the power of this approach to reconstruct paleoclimatic parameters, to track paleo-hydrological changes, and to a certain extent, to detect shift of ancient atmospheric circulation becomes immediate apparent. Andersen et al. [79] found D-enriched *n*-alkane and isoprenoids in some intervals of Miocene Messinian sediments from the Mediterranean Sea, and interpreted the large fluctuation (up to 160‰) of δD values of biomarkers as a reflection of intense evaporation of lake water and changes of relative humidity during that period. Similarly, Liu and Huang [100] correlated the large variation of *n*-alkane δD changes for the past 130,000 years on the Chinese Loess Plateau with aridity through the control of precipitation. A long core study of 50‰ δD shift of leaf wax *n*-alkanes in Miocene sediments was attributed to the effect of changes in precipitation and evaporation [101]. Barker et al. [102] stated that the sediment *n*-acid δD changes in African Lake Malawi represented major shifts in precipitation and evaporation at the century-to-millennial scale.

One of the major advantages of using compound-specific hydrogen analysis is that the source of a particular compound is known and the apparent hydrogen fractionation between the molecular δD and source water δD can be obtained empirically. Thus, δD from different compounds of the same sample can be used to track different environmental parameters. For example, using short carbon chain behenic acid (C₂₂ *n*-acid) and long carbon chain fatty acids (C₂₄–C₃₂ *n*-acids), Hou et al. [89] have inferred lake water δD (believed to be controlled primarily by temperature) and the relative humidity of growing season (tied to precipitation), respectively. Similarly, Mügler et al. [103] have shown that *n*-alkanes from aquatic and terrestrial sources can be used to track the evaporation of lake water and atmospheric precipitation separately; thus the difference between aquatic and terrestrial *n*-alkanes can be applied to differentiate wet/dry sedimentary conditions.

A recent exciting application of plant molecular hydrogen isotope data is to infer the long-term dynamic of ancient air mass that was previously off the limit of study using other geological approaches. As the dynamic range of atmospheric transport is largely controlled by the temperature gradient [104,105], which strongly influences isotopic compositions of precipitation [106–108], δD values from plant waxes may carry the signal of source, transport, and change of ancient air masses. Jacob et al. [109] attributed the change of humidity during the Younger Dryas and at the end of the Last Glacial Maximum to the alteration of atmospheric system that was controlled by the Inter Tropical Convergence Zone (ITCZ) in Northern Brazil. This finding is in contrast with the interpretation based upon data from Africa [110] which have suggested that the increase of precipitation pattern change, as recorded in δD values of *n*-C₂₉ alkanes at the beginning of the Younger Dryas period, was not due to the positional change of ITCZ. Using δD of *n*-C₁₇ and *n*-C₂₉ alkanes from Arctic

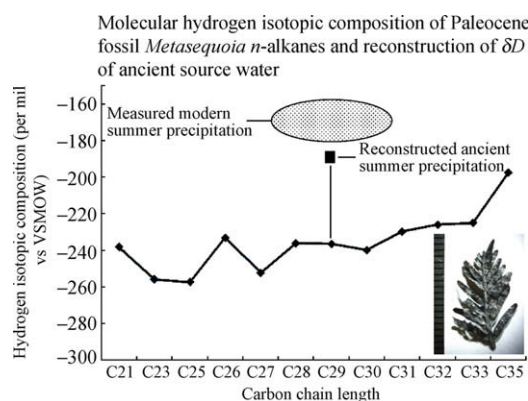


Fig. 5. Molecular hydrogen isotope composition of *n*-alkanes from late Paleocene *Metasequoia* leaves (inset) and reconstruction of the ancient source water δD for the Arctic area based on experimentally determined apparent hydrogen isotope fractionation of -62‰ (Yang et al. unpublished data). The range of modern D/H values of summer growing season of the same area is plotted (shade area) for comparison.

sediments at the Paleocene–Eocene Thermal Maximum (PETM), Pagani et al. [10] have proposed that a decrease in rainout during moisture transport from lower latitudes at the onset of PETM has increased moisture delivery to the Arctic during the global warming period. Recently, applying experimentally calculated $\epsilon_{\text{lipid-water}}$ values to the hydrogen isotope compositions of *in situ* *n*-alkanes from extraordinarily preserved deciduous conifer fossils from the Arctic, Yang et al. (unpublished data) estimated D/H values of summer growing season precipitation during Late Paleocene to Middle Eocene at the high Arctic (Fig. 5). They have found that these similar or slightly D-depleted values, relative to those of the present day, imply that considerable amount of high latitude summer precipitation in this region was supplemented by moisture derived from regional transpiration of the vast polar forests established during global warming of the early Cenozoic.

7. Conclusion and perspectives

Molecular hydrogen isotope analysis in fossil and living plants represents a new and active research frontier that holds great potentials for studies in plant biology, paleobotany, plant paleoecology, and paleoclimatology. Compared with carbon isotope analysis, the large hydrogen isotopic fractionation offers a greater opportunity, as well as technical challenges, for geobiological applications. Facilitated by the recent development and constant improvement of online isotope ratio monitoring gas chromatography/thermal conversion/mass spectrometry, compound-specific δD data have enhanced our knowledge on major paleoclimatic events since Cenozoic, such as the PETM [10], the Miocene C_4 plant expansion [101], the Last Glacial Maximum, and the Younger Dryas [109,110]. Current studies employ samples from across different continents: America [88], Asia [100,101], Africa [102,110], Europe [52], and South America [109], ranging from tropics [111] to the Arctic [10].

The challenge laid ahead is to better understand the multiple factors that control hydrogen isotope fractionations during the incorporation of hydrogen into plant molecules and the diagenetic alteration of hydrogen isotope signals in the geological record. A combination of field-based researches and empirical studies under controlled laboratory conditions is likely to offer insights into the influence on δD variations in different plant molecules. The compilation of large global database on δD of a particular molecule (such as *n*-alkane [6]) may provide quantitative correlations between δD and specific environmental factors, such as salinity, temperature, relative humidity, and precipitation. It is clear that a more accurate reconstruction of paleoenvironmental conditions can be achieved by applying taxon-specific molecular hydrogen isotope fractionations to *in situ* molecules obtained from identifiable fossil material in conjunction with $\delta^{13}\text{C}$ data as well as other proxies derived from the traditional paleontological research.

Acknowledgements

This work was supported in part by the Pilot Project of Knowledge Innovation, CAS (KZCX2-YW-105), the CAS/SAFEA International Partnership Program for Creative Research Teams, the Major Basic Research Projects (Grant No. 2006CB806400), the National Natural Science Foundation of China (Grant Nos. 40402002 and 40872011), the American Chemical Society Petroleum Research Funds, a NASA Rhode Island Space Grant, and a Bryant University Summer Research Fund. We thank Robert Patalano and Liang Xiao for preparing diagrams and references. This paper is the contribution 200902 for the Laboratory of Terrestrial Environment at Bryant University.

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