HYDROGEN ISOTOPIC COMPOSITION OF FATTY ACIDS, STEROLS, AND PHYTOL: AUTOTROPHIC VS. HETEROTROPHIC PRODUCTION

Y. Chikaraishi

Institute of Low Temperature Science, Hokkaido University, Japan
Japan Agency for Marine-Earth Science and Tecnology, Japan

Introduction

Stable hydrogen isotopic composition (δD) of lipids has been increasingly employed as a hydrological proxy in the study of paleoenvironments and paleoecosystems over a wide range of geological timescales (see the review by Sachse et al., 2012). This is based on empirical observations that the δD values of lipids in algae and terrestrial plants well record those of ambient water and the condition of environments (e.g., salinity, nutrients, light intensity, humidity, etc.) where the algae and plants grew. However, a number of isotopically distinct pools of hydrogen can be utilized during lipid biosynthesis in algal and plant cells: for instance, proton (H+, ca. 0‰) is derived from cell water, and hydride (H−, ca. −60‰ to −100‰) is derived from NADPH that produced by multiple redox reactions in photosynthesis and secondary metabolisms (see the review by Hayes, 2001; Schmidt et al., 2003). Accordingly, the relative contribution among the isotopically distinct pools of hydrogen to lipid biosynthesis and its environmental variability will be important to determine the δD values of lipids in algae and plants. Indeed, the δD values of lipids are frequently elevated by over 50‰ when the algae and plants are in the stage of heterotrophic production (e.g., Sessions, 2006), which can be explained by that NADPH pool derived from metabolism (that may be used a lot for heterotrophic production) is weakly depleted in D (e.g., −100‰) as compared with that derived from photosynthesis (e.g., −600‰, for photosynthetic production).

To improve our understanding of the δD values of lipids, in the present study we investigated a model alga, the chlorophyte *Chlamydomonas reinhardtii*, which can grow either photosynthetically or heterotrophically. *C. reinhardtii* was cultured photosynthetically in water (δD = −55‰) under a 14-hour light/10-hour dark cycle for 2 weeks, or heterotrophically in five isotopically distinct water (δD = −283‰, −55‰, +45‰, +161‰, and +479‰) and acetic acid (δD = −140‰) as alternative source of hydrogen under a 24-hour dark for 2 weeks. The δD values of fatty acids, sterols, and phytol were determined in these cultivated cells.

Results and Discussion

In the photosynthetic culture, the δD values of fatty acids were widely distributed from −231 to −67‰, and of ergosterol, 7-dehydroporiferasterol, and phytol were −287, −260, and −364‰, respectively. Although there is a large variation in the δD value among the lipids, the δD values observed are in a common trend for algae and plants, as fatty acids are less depleted in D as a result of acetogenic pathway and subsequently enriched in D during unsaturation, sterols are much depleted in D as a result of either the methylerythritol phosphate (MEP) or the mevalonic acid (MVA) pathway, and phytol is much strongly depleted in D as a result of hydrogenation to the product of the MEP or the MVA pathway (e.g., Chikaraishi et al., 2004a, 2004b).

On the other hand, in the heterotrophic cultures, the δD values of these lipids are positively correlated to those of ambient water, but interestingly, with gentle (or flat) slopes ranging from...
0.10 to 0.36 and correlation coefficients ($R^2$) ranging from 0.94 to 0.99 (Fig. 1). These gentle slopes observed in this study have never been reported in photoautotrophic cultures of algae: for example, steeper slopes have generally been observed in cultures of coccolithophorids (0.73-0.75 for alkenones, Englebrecht and Sachs, 2005) and chlorophyte (e.g., 0.72-0.95 for palmitic acid; Zhang and Sachs, 2007). These gentle slopes can be simply explained by a small contribution of ‘ambient water-derived hydrogen’ but a large contribution of ‘acetic acid-derived hydrogen’ to the biosynthesis of the lipids in the heterotrophic cultures of *C. reinhardtii*. Thus, unlike in photosynthetically-produced lipids, in which lipid biosynthesis employs ambient water as the primary source of hydrogen, substrates (acetic acid in the present study) are major sources of hydrogen for heterotrophically-produced lipids.

Based on these results, we conclude that knowing the balance between photosynthetic and heterotrophic productions and its environmental variables can be useful for great enhancing of the value of isotope evidences in application studies such as for tracing the delivery of organic compounds and for reconstructing the paleoenvironments and paleoecosystems.

![Figure 1](image.png)

**Figure 1** Relationships between the δD values of lipids (i.e., fatty acids, sterols, and phytol) and those of ambient water in heterotrophic cultures of *C. reinhardtii*.

**References**

Chikaraishi et al. (2004a) *Phytochemistry* 65, 1369-1381.  
Chikaraishi et al. (2004b) *Phytochemistry* 65, 2293-2300.  