Short communication

D/H exchange in C–H bonds of fatty acids: Implication for geographical discrimination of food materials

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ABSTRACT

The stable isotope analysis of non-exchangeable hydrogen in food materials has been believed as a powerful tool for tracing geographical origins and delivery of the materials. However, little information is available for the D/H exchanges even in hydrophobic molecules, which leads to potential uncertainty on the observed results. To further evaluate the reliability of hydrogen isotopes of organic molecules, we examined the isotope exchangeability in fatty acids within phospholipid bilayers exposed to D2O for 12 h. The results indicate that the isotope ratio is clearly increased during all examines (e.g., 93–328‰ in 50% D2O), in which its magnitude depends on type of fatty acids and state of bilayers. However, these results also indicate very clearly that the observed exchange rate is negligible if samples are exposed to natural abundance of deuterium (0.0156%) in natural and laboratory environments before/during analysis.

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1. Introduction

The stable isotope analysis of common elements (hydrogen: D/H, carbon: 13C/12C, nitrogen: 15N/14N, oxygen: 18O/16O, and sulphur: 34S/32S) has been widely employed as potential tools (or proxies) for tracing the geographical origins and delivery of food materials (e.g., Kelly, Heaton, & Hoogewerff, 2002; Nakashita et al., 2008; Suzuki, Chikaraishi, Ogawa, Ohkouchi, & Korenaga, 2008). In particular, the hydrogen isotopic composition of hydrogen in C–H bonds on hydrophobic molecules such as n-alkanes and fatty acids has been believed as a powerful tool in a many cause of studies, because it has the largest difference in relative weight between two stable isotopes (H and D) and resulting variation occurs as much as 600‰ in nature (as δD values, relative to V-SMOW) (e.g., Chikaraishi, Suzuki, & Naraoka, 2004; Zhang, Sachs, & Marchetti, 2009). For example, the isotopic composition of rainfall (i.e., hydrogen source of foods) shows unique variation with latitude and altitude, ranging from −430‰ to +50‰ (Database of Hydrogen Isotopic Composition of Rainfall, by GNIP 2010). Such a large variation is basically inherited into the hydrogen found in natural organic materials such as vegetables and meats as well as organic compounds in those (e.g., Cormie, Schawarcz, & Gray, 1994; Huang, Shuman, Wang, & Webb, 2002; Sachse, Radke, & Gleixner, 2004). So far, previous studies have generally assumed as that D/H exchange of hydrogen in C–H bonds on hydrophobic molecules is unimportant in natural environments and during chemical analyses in laboratories. However, very little is known about D/H exchange in hydrophobic molecules within natural and laboratory environments. In fact, several previous studies have reported variable D/H exchange potential of C–H bonds on dicarboxylic acid (Fuller & Huang, 2003), hydrocarbons (Sessions, Sylva, Summons, & Hayes, 2004), and ketones (Wang, Sessions, Nielsen, & Goddard, 2009a, 2009b). Thus, further evaluation of the reliability of hydrogen isotope analysis of H–C bonds in organic compounds (e.g., fatty acids) is necessary before applying it as a critical tool (or proxy) in the types of studies outlined above. Therefore, in this study we examine D/H exchange on fatty acids in a phospholipid bilayer of pseudo cell membranes exposed to 0–50% D2O at either room temperature or 85°C for 12 h. Phospholipid bilayers are a good model in this context because fatty acids occur abundantly in this form within natural environments (e.g., as membranes for both plant and animal cells). Natural phospholipid bilayers consist of various kinds of fatty acids (with a range of carbon chain lengths and degrees of saturation) to control liquidity with respect to the temperature of the environment. Here we examine three types of fatty acids: C18 saturated (stearic), C18 monosaturated (oleic), and C12 saturated (lauric).
2. Experimental

2.1. D/H exchange of phospholipids

Three types of lipid bilayers, including distearoyl (DLPC), distearoyl phosphatidylcholine (DSPC), and dioleoyl (DOPC) of L-α-phosphatidylcholine, were prepared by a simple hydration. Briefly, 120 g of phospholipid powder was dissolved in 8 ml of chloroform. The solution was concentrated by rotary evaporation and then thinned to form a phospholipid film. The film was dissolved in 40 ml of phosphate buffer adjusted to pH = 7 and then dispersed by vortex mixer at the phase transition temperatures (Table 1). After ultrasonic irradiation, uniform and spherical phospholipid bilayers (1–10 μm in diameter) were obtained.

Phosphate buffer with 99.9% D2O was added into 5 ml of the phospholipid bilayer and adjusted to D2O concentrations of 0% (control without D2O), 1.0%, 6.3%, 12.5%, 25.0%, and 50.0%. After 12 h exposure at either room temperature or 85 °C, and with or without stirring (Table 1), the solvent was removed by freeze-drying. Since DSPC is precipitated at room temperature as a gel state, one of the DSPC samples was stirred to hold uniformity and another was heated at 85 °C to hold liquid crystalline state.

2.2. Hydrogen isotope analysis of fatty acids

Fatty acids were extracted by alkali hydrolysis and the hydrogen isotopic composition of the extracted fatty acids was determined by thermal conversion elemental analyzer/isotope ratio mass spectrometer (TCEA/IRMS). Briefly, the phospholipid bilayer was hydrolysed with 0.5 M KOH (aqueous) at 75 °C for 12 h to obtain the fatty acid fraction, and then the pH of the solution was changed to <1.0 by adding HCl. The fatty acids were extracted with hexane and then dried under a gentle stream of nitrogen gas. Hydrogen isotope analysis of the fatty acids was performed by TCEA/IRMS using a Flash EA 1112HT TCEA coupled to a Delta V Advantage IRMS through a Conflo III interface (Thermo Fisher Scientific). Approximately 0.3 mg of fatty acid was analysed by TCEA/IRMS. To minimise memory effects, blank silver capsules were analysed by each sample run. The hydrogen isotopic composition of fatty acids is expressed with δD notation relative to the V-SMOW international standard (Eq. (1)) and normalised with polyethylene (δD = −100.3‰, IAEA) and hexatriacontane (δD = −246.7‰, Indiana University).

\[ \delta D = \frac{\delta D_{\text{FA}} - \delta D_{\text{V-SMOW}}}{\delta D_{\text{V-SMOW}}} \times 1000(\%) \]

where \( R \) is the absolute D/H ratio of fatty acid (\( R_{\text{FA}} \)) or V-SMOW (\( R_{\text{V-SMOW}} = \sim 1.6 \times 10^{-4} \)). To find very weak deuterium-enrichment, the δD notation shows the relative difference in D/H ratio between the fatty acid and an international standard, in units of per mil (‰, parts per thousand). Based on replicate analysis (N = 5), a mean deuterium-enrichment (\( \Delta D \), Eq. (2)) of fatty acid is reported.

\[ \Delta D = \delta D_{\text{change}} - \delta D_{\text{control}} \]

where \( \delta D_{\text{change}} \) and \( \delta D_{\text{control}} \) represent the δD values of fatty acid exposed with and without D2O, respectively. The use of \( \Delta D \) values minimises D/H exchange during the experimental procedure (e.g., alkali hydrolysis).

3. Results and discussion

3.1. D/H exchange

Deuterium-enrichments of fatty acids are summarised in Table 2. In general, the \( \Delta D \) values are positive for all cases: \( \Delta D \) increases with an increase of D2O concentration and is much larger for gel states than for liquid crystalline states. The gel state of DSPC with stirring shows the largest value, up to more than 300‰. For samples exposed to 50% D2O, D/H exchange is increased in the following order: DSPC stirred at room temperature (\( \Delta D = 329\% \)) > DSPC stood at room temperature (\( \Delta D = 208\% \)) > DOPC stood at room temperature (\( \Delta D = 138\% \)) > DLPC stood at room temperature (\( \Delta D = 115\% \)) > DSPC stood at 85 °C (\( \Delta D = 93\% \)). These results show that hydrogen isotopes are clearly exchanged in fatty acid C–H bonds and that the magnitude of the exchange may depend on the type of fatty acid (chain length and unsaturation degree) and the state of bilayers (gel versus liquid crystal). The observed D/H exchange on C–H bonds of hydrophobes is consistent with the results of previous studies using other compounds (e.g., Fuller & Huang, 2003; Sessions et al., 2004; Wang et al., 2009b).

However, as an important finding, such D/H exchange cannot be detected if bilayers are exposed to water with natural D/H abundance (D/H = \sim 1.6 \times 10^{-4}) during analytical procedures in laboratories. In fact, it is roughly estimated that hydrogen isotopic composition is changed by up to a maximum of 0.01‰ (i.e., the change in D/H is \sim 3 \times 10^{-6}) when exposed to water with a deuterium enrichment of only 100‰ in the same condition described above. Considering the general analytical error of 3–7‰ of the isotope analysis, we conclude that the D/H exchange is substantially small or negligible in natural environments.

For DSPC (stearic acid), the D/H exchange increased in the following order: stirred at room temperature > stood at room temperature > stood at 85 °C. On the other hand, among three types of fatty acids (stearic, oleic, and lauric acids), the D/H exchange increased in the following order: oleic acid > stearic acid > lauric acid, although the exchange difference is smaller than that in the experiments for gel state. These results suggest that the magnitude of D/H exchange depends on the type of fatty acid (i.e., chain length and unsaturation degree) and the state of bilayers (gel versus liquid crystal). The specific mechanisms responsible for these differences are not known, but may relate to the membrane permeability of phospholipid bilayers.

4. Summary

In this study, we evaluated the D/H exchange on fatty acids in phospholipid bilayers of pseudo cell membranes exposed to 0–50% D2O. The results show that hydrogen isotopes are clearly exchanged in fatty acid C–H bonds and that the magnitude of the exchange may depend on the type of fatty acid (chain length and unsaturation degree) and the state of bilayers (gel versus liquid

| Table 1 | Character of phospholipid bilayer and conditions of D/H exchange. |
| --- | --- | --- | --- |
| Material | Constituent | Phase transition temperature (°C) | Condition of D/H exchange |
| No. 1 | DSPC | Stearic acid (18:0) | 55 | Temperature (°C) |
| No. 2 | DSPC | Stearic acid (18:0) | 55 | Stir |
| No. 3 | DSPC | Stearic acid (18:0) | 55 | State of lipid bilayer |
| No. 4 | DLPC | Lauric acid (12:0) | 0 | 25 |
| No. 5 | DOPC | Oleic acid (18:1) | –22 | 25 |
| | | | | 25 | On | Gel |
| | | | | 25 | Off | Gel |
| | | | | 85 | Off | Liquid crystal |
| | | | | 25 | Off | Liquid crystal |
| | | | | 25 | Off | Liquid crystal |
crystal). Variations in exchange may be related to the membrane permeability of phospholipid bilayers. However, the magnitude of the observed D/H exchange is sufficiently small if the bilayers are exposed to water with a natural abundance of deuterium (0.0156%) in natural and laboratory environments. The exchange is estimated to be, at maximum, only $\frac{0.01}{C24}$‰ if the bilayers are exposed to water with an isotopic difference of 100‰.

Thus, the hydrogen isotopes of fatty acids certainly exchange with those of ambient water but this exchange is not substantial when they are exposed to water with a natural abundance/variation in deuterium (i.e., in the natural environment and during laboratory analysis). This finding suggests that the isotope signatures of hydrogen in C–H bonds on hydrophobic molecules are useful as a potential tool in various studies that identify the geographical origin and transport of food materials.

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References


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<tr>
<th>Constituent</th>
<th>State of phospholipids</th>
<th>Concentration of heavy water (%)</th>
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<tbody>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>No. 1</td>
<td>Stearic acid</td>
<td>Gel state</td>
</tr>
<tr>
<td>No. 2</td>
<td>Stearic acid</td>
<td>Gel state</td>
</tr>
<tr>
<td>No. 3</td>
<td>Stearic acid</td>
<td>Liquid crystalline state</td>
</tr>
<tr>
<td>No. 4</td>
<td>Lauric acid</td>
<td>Liquid crystalline state</td>
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<tr>
<td>No. 5</td>
<td>Oleic acid</td>
<td>Liquid crystalline state</td>
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