Carbon isotopic difference of saturated long-chain n-fatty acids between a terrestrial and a marine sediment

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Stable carbon isotopic compositions of individual n-fatty acids ranging from C₂₀ to C₃₀ in a terrestrial (riverine) and a marine (pelagic) sediment were determined using gas chromatography/isotope ratio monitoring mass spectrometry (GC/IRMS). These fatty acids in a terrestrial sediment at the Ohtsuchi river in northern Honshu Island, Japan, showed $\delta^{13}\text{C}$ values (from -35 to -32‰ relative to PDB) lighter than that of the total organic carbon (TOC; -26.4‰) by about 6 to 9‰. The $\delta^{13}\text{C}$ values from a marine sediment in the Pacific Ocean ca. 500 km off the coast of Honshu Island varied from -31 to -26‰, which were also lighter than that of the TOC (-20.5‰) by 6 to 9‰. The $\delta^{13}\text{C}$ values of n-fatty acids ranging from C₂₀ to C₃₀ in the marine sediment were heavier by about 5 to 6‰ than those at the corresponding carbon numbers in the terrestrial one. Isotopically, the marine n-fatty acids were clearly distinguished from the terrestrial ones. These results suggest that long-chain n-fatty acids in marine sediment were not derived from land higher plant but from marine organisms, although long-chain n-fatty acids ($\geq\text{C}_{20}$) in marine sediments have often been used to assess terrestrial material contribution to the ocean.

INTRODUCTION

Estimation of the proportions of terrestrial and marine organic materials in marine sediments is very important for the better understanding of the global carbon cycle. Discrimination of organic matter between two sources has often been conducted by ratio of total organic carbon (TOC) to nitrogen content (C/N) (Stevenson and Cheng, 1972; Muller, 1977) and isotopic composition of TOC (e.g. Degens, 1969; Sackett, 1987). The C/N ratios in most marine sediments have lower values (<ca. 8 by weight) than those of terrestrial ones (C/N > ca. 10). Several studies clarified a systematic change in $\delta^{13}\text{C}_{\text{TOC}}$ values of sediments from rivers to bays and to open sea (Sackett and Tompson, 1963; Hunt, 1970; Wada *et al.*, 1987). The $\delta^{13}\text{C}_{\text{TOC}}$ values of open sea sediments are relatively constant (ca. -20‰ on average relative to PDB) and are heavier than those of terrestrial ones (ca. -26‰ on average). Accordingly, their values have been used to estimate the proportions

of terrestrial and marine organic contributions in marine sediments. In molecular levels, organic compounds derived from lignin, suberin and cutin have been used as indicators of terrestrial materials (Hedges and Parker, 1976; Goni and Hedges, 1990), since they are characteristically contained in terrestrial higher plants (e.g. Kolattukudy, 1980).

Fatty acids, major components of lipids in sediments, have been difficult to determine their sources either marine or terrestrial, because they occur very widely in nature. Among saturated fatty acids, hexadecanoic (n-C₁₆) and octadecanoic acid (n-C₁₈) are abundant and may have originated from both marine and terrestrial biological materials. However, long-chain n-fatty acids ($\geq\text{C}_{20}$) in marine sediments have been often used by many investigators (e.g. Simoneit, 1978; Brassel, 1993) to infer terrestrial higher plant input to the sediment through riverine or by aeolian transport, since they are usually predominant components in higher plant wax, suberin and cutin (e.g. Kolattukudy, 1980). Simoneit (1977) used the long-chain n-fatty

acid as terrestrial indicators in the Black Sea sediments, where the major input mechanisms were potamic transport. Prahl *et al.* (1989) used the abundance of long-chain n-fatty acids to estimate the proportion of terrestrial to marine TOC in the sediments of northeastern tropical Pacific Ocean ($0^{\circ}57.2' \text{ N}$, $138^{\circ}57.3' \text{ E}$), where the fatty acids were assumed to be land-derived by long-range aeolian transport because of no major continental landmass nearby. However, the origin and mixing ratio of terrestrial and marine long-chain n-fatty acids in marine sediments have not been understood well. For instance, Volkman *et al.* (1980) pointed out the resemblance in molecular distribution of long-chain n-fatty acids between intertidal sediment and marine organism (diatom).

Individual stable carbon isotopic compositions of abundant fatty acids (e.g. n-C₁₆ and n-C₁₈) have been reported on various living organisms and sediments (Parker, 1962, 1964; Vogler and Hayes, 1980; Monson and Hayes, 1980, 1982) by isolating each compound using gas chromatography (GC) and subsequently oxidizing to CO₂. Those of long-chain n-fatty acids ($\geq \text{C}_{20}$), however, have never been studied because of their relatively low abundances in sediments. In this study, we have determined the isotopic compositions of individual n-fatty acids at even carbon numbers ranging from C₂₀ to C₃₀ in terrestrial (riverine) and marine

(pelagic) sediments using a new technique of gas chromatography/isotope ratio monitoring mass spectrometry (GC/IRMS) (Matthews and Hayes, 1978; Hayes *et al.*, 1990). We report their isotopic features and discuss the sources of long-chain n-fatty acids in marine sediments.

EXPERIMENTAL

The terrestrial sample (OR3) used for this study was a riverine surface sediment which came from the Ohtsuchi river in Iwate-prefecture, northern Honshu Island, Japan. The marine sample (SR72) was a 36 cm core from the northwestern Pacific Ocean ($39^{\circ}11.4' \text{ N}$, $147^{\circ}06.2' \text{ E}$; about 500 km east off Honshu Island, or 250 km east from the Japan trench: 5360 m in depth) (Fig. 1). It was composed mainly of carbonate-free, diatom-rich siliceous matter. After subjected to the freeze-dry procedure, the both sediment samples were treated with 6 M HCl to remove carbonate carbon. The TOC contents and C/N ratios were determined using a CHN analyzer (Yanaco, MT-3), and $\delta^{13}\text{C}_{\text{TOC}}$ values by the method of CuO-combustion in quartz tubes (Boutton *et al.*, 1983) followed by the measurement of isotopic ratios using a double inlet mass spectrometer (Finnigan MAT delta S).

For fatty acid analysis, the SR72 sediment sample was a mixture taken from the part of 4 to

Fig. 1. Sampling sites of terrestrial (OR3) and marine (SR72) sediments (see text).

34 cm of the core. The samples were saponified with 0.5 M KOH in methanol by refluxing. After neutral organic compounds were removed, the fatty acid fraction was obtained and divided into two sub-fractions. One fraction was converted to trimethylsilyl derivatives, and analyzed by GC or gas chromatography/mass spectrometry (GC/MS). Another fraction was treated with 14% BF₃/MeOH to convert to the methyl ester derivatives, which were subsequently developed by a AgNO₃-silica gel (5% w/w) column to isolate the saturated fatty acid fraction for the stable carbon isotope analysis by a GC/IRMS. The system used was a Finnigan MAT delta S mass spectrometer combined with a Varian 3400 gas chromatograph through a capillary combustion furnace (CuO/Pt).

Detailed analytical procedures and isotopic correction method will be reported elsewhere (Naraoka *et al.*, 1994). Briefly, three n-alkanes as isotopic internal standards were coinjected with the samples. Isotopic correction was carried out for the fatty acids with one additional carbon atom using the $\delta^{13}\text{C}$ values of methanol (−37.4‰) in BF₃/methanol. For standard n-fatty acids, analytical accuracy (deviation of the corrected $\delta^{13}\text{C}$ values determined by GC/IRMS from those determined by the conventional method) was $\pm 0.3\%$ for the average of several analyses. Precision (standard deviation (*s*) defined as $s = (\sum(x_i - x_{\text{ave.}})^2 / (n - 1))^{1/2}$; x_i : measured values, $x_{\text{ave.}}$: averaged value, *n*: number of analyses) was within 0.3% (*s*).

RESULTS AND DISCUSSION

TOC contents, C/N ratios and carbon isotopic compositions of TOC of the sediments

The OR3 sediment had TOC content 3.19 wt%, and C/N ratio 10.8 with the $\delta^{13}\text{C}_{\text{TOC}}$ of −26.4‰. For the SR72 sediment, these values were determined for every 2 cm through the core; the TOC content 0.66 (0.56 to 0.81) wt%, C/N ratio 7.1 (6.6 to 7.9), and $\delta^{13}\text{C}_{\text{TOC}}$ of −20.5 (−20.8 to −20.1)‰ on average used for this study. The ranges of measured values are shown in parentheses. The C/N ratios and $\delta^{13}\text{C}_{\text{TOC}}$ values indi-

cate that bulk organic matter in the OR3 and SR72 sediments have typical characteristics of terrestrial and marine sediment, respectively.

Molecular distributions of fatty acids and other terrigenous compounds

Relative abundances of major fatty acids in the sediments are shown in Fig. 2 (as the content of n-C₁₆ = 100). The contents of the most abundant fatty acid (n-C₁₆) are quite different between OR3 (47 $\mu\text{g/g}$ dry sediment) and SR72 (4 $\mu\text{g/g}$ dry sediment). However, the relative abundances of saturated n-fatty acids ranging from C₁₄ to C₃₀ are not very different in both samples. The abundance pattern exhibits maxima in two regions (n-C₁₆, and n-C₂₂ (OR3) or n-C₂₆ (SR72)). This bimodal distribution has been commonly observed in marine sediments (e.g. Simoneit, 1978; Nishimura and Baker, 1987) and sedimentary rocks (e.g. Grimalt *et al.*, 1990). Furthermore, both have the predominance of even carbon numbered fatty acids. For long-chain n-fatty acids, the abundance ratio of even to odd carbon-numbered molecules (defined here as $(n\text{-C}_{22} + n\text{-C}_{24} + n\text{-C}_{26} + n\text{-C}_{28}) / (n\text{-C}_{21} + n\text{-C}_{23} + n\text{-C}_{25} + n\text{-C}_{27})$, where n-C_m represents the contents of n-C_m fatty acid) are 3.4 and 4.1 for the OR3 and SR72 sediments, respectively. Therefore, it is impossible to determine whether the long-chain n-fatty acids in the SR72 sediment are terrestrial and/or marine in origin only by their relative abundances.

In addition to the dominance of saturated n-fatty acids, ω -hydroxymonocarboxylic and α,ω -dicarboxylic acids ranging from C₁₄ to C₂₆ at even carbon numbers are prominent components in the OR3, but less in the SR72 sediment (Fig. 2). These acid mixtures are characteristic of cutin and suberin compounds from land higher plants (Kolattukudy, 1980). Unsaturated (C_{18:1}) ω -hydroxymonocarboxylic and α,ω -dicarboxylic acid may be used to distinguish terrestrial organic contribution from marine one in the sediments. Furthermore, alkaline CuO oxidation of the sediments revealed that lignin-derived compounds such as vanillyl and syringyl phenols with aldehyde, ketone and carboxyl functional groups were more

Fig. 2. Relative abundances of fatty acids in the OR3 and SR72 sediments. Each fatty acid is normalized to the most abundant fatty acid, hexadecanoic acid ($n\text{-C}_{16}$) (=100). The term, m , represents the carbon number of fatty acid. $n\text{-C}_m$ = saturated n -fatty acid, $n\text{-C}_{m:1}$ = monounsaturated n -fatty acid, $\omega\text{-OH-C}_m$ = ω -monohydroxy saturated fatty acid, $\omega\text{-OH-C}_{m:1}$ = ω -monohydroxy monounsaturated fatty acid, C_m = α,ω -dicarboxylic saturated n -fatty acid, $\text{diacid } C_{m:1}$ = α,ω -dicarboxylic monounsaturated n -fatty acid.

abundant in the OR3 (ca. 1000 $\mu\text{g/g}$ dry sediment) than in the SR72 (ca. 10 $\mu\text{g/g}$ dry sediment) (Ishiwatari, unpublished result). Terrestrial contribution of organic matter must be low in the SR72 sediment.

Carbon isotopic compositions of individual long-chain n -fatty acids in the OR3 and SR72 sediment, and possible sources of the long-chain n -fatty acids in the SR72 sediment

Figure 3 shows the $\delta^{13}\text{C}$ values of individual n -fatty acids at even carbon numbers in the two sediments. The $\text{C}_{20}\text{--C}_{30}$ fatty acids in the OR3 sediment have lighter $\delta^{13}\text{C}$ values (from -35 to -32‰) than that of TOC (-26.4‰) by 6 to 9 ‰ . The result is consistent with the previous studies that $n\text{-C}_{16}$ and $n\text{-C}_{18}$ fatty acids including saturated and unsaturated structures have usually lower $\delta^{13}\text{C}$ values than those of total organisms (Parker, 1962, 1964). On the other hand, these $\delta^{13}\text{C}$ values in the SR72 sediment vary from -31 to -26‰ ,

which are also lighter than that of TOC (-20.5‰) by 6 to 10 ‰ . The $\delta^{13}\text{C}$ values of n -fatty acids ranging from C_{20} to C_{30} in the SR72 sediment are heavier by 5 to 6 ‰ than those of the corresponding carbon numbers in the OR3 sediment. The result indicates that the marine n -fatty acids can be distinguished clearly from those in terrestrial ones isotopically.

Saturated fatty acids in organisms are synthesized through elongation of acetyl-CoA (e.g. Hitchcock and Nichols, 1971). Or it is suggested that exogenous fatty acids are incorporated into phospholipids (Silbert *et al.*, 1968; Monson and Hayes, 1980). In any case, the $\delta^{13}\text{C}$ value of n -fatty acid in sediment depends on the isotopic composition of carbon source where the fatty acid is biosynthesized. Therefore, the $\delta^{13}\text{C}$ value differences of individual n -fatty acids between the OR3 and SR72 sediments are due to (i) the difference in primary producers, i.e. terrestrial or marine carbon fixers under different conditions,

Fig. 3. Carbon isotopic compositions of individual saturated n-fatty acid (corrected from those of methyl ester derivatives) ranging from C₂₀ to C₃₀ at even carbon numbers in the OR3 and SR72 sediments. The $\delta^{13}\text{C}$ values were obtained by duplicate and triplicate analyses for OR3 and SR72 sediments, respectively. Error bars represent standard deviations $2s$ ($\pm s$).

(ii) the isotopic shift by biological reworking and early diagenesis during transportation and deposition, and/or (iii) the difference of age dependent environmental conditions such as temperature and partial pressure of atmospheric CO₂ during photosynthetic fixation. The alternative (iii) could arise from the reason that the SR72 is *not young* sediment, which deposited from *ca.* 20 to 150 thousand years ago, because sedimentation rate for the SR72 sediment is quite small (2.3 mm/kyr) (Narita *et al.*, 1990). However, the $\delta^{13}\text{C}$ values of bulk organic carbon are practically constant ($-20.5 \pm 0.3\text{‰}$) throughout the sediment core. It suggests that drastic environmental change, which had caused the isotopic shift of fatty acids by *ca.* 5‰, has not occurred in this region during the past 150 thousand years. Therefore, the last alternative is much less plausible.

For the alternative (ii), the isotopic shift of fatty acids by biological reworking and diagenesis is not well known. Diagenesis involving decarboxylation of carboxyl groups may make the carbon isotopes of total fatty acids heavier, because biologically-formed carboxyl groups are usually depleted in ^{13}C relative to their corresponding molecular average within 10‰ (Vogler and Hayes, 1980; Monson and Hayes, 1982). So decarboxy-

lation of fatty acids could contribute to increase the $\delta^{13}\text{C}$ value by 1‰ or less as a whole molecule, even if the reaction is followed by oxidation to produce other shorter chain fatty acids. Furthermore, it is important to realize that long-chain n-fatty acids ($>\text{C}_{20}$) are not utilized well by organisms because they are not better primary substrate as energy sources in various living organisms than abundant fatty acids of C₁₄, C₁₆ and C₁₈ (Nishimura and Baker, 1987). These considerations may indicate that the $\delta^{13}\text{C}$ values of long-chain n-fatty acids in the two sediments have not little changed in these processes of alternative (ii).

The alternative (i) is most plausible by the following reason. The n-fatty acids in the OR3 and SR72 sediments show equally lighter $\delta^{13}\text{C}$ values than that of TOC by about 6 to 9‰. The isotopic difference could reflect the isotope difference of primary substrates depending on the terrestrial or marine assimilation process. It is known that $\delta^{13}\text{C}$ values of total lipid extracts for various plant and plankton species are lighter than those of TOC by 5‰ on average (Degens, 1969; Deines, 1980). Therefore, we may conclude that long-chain n-fatty acids (C₂₀–C₃₀) in the SR72 sediment are not derived from land higher plants but from organisms in marine environment. Our

finding indicates the necessity of reconsideration of reliability of these compounds as an indicator of terrestrial organic matter.

As mentioned above, Volkman *et al.* (1980) reported the resemblance of molecular distribution of long-chain fatty acids between intertidal sediment and diatom culture. Recently, some seagrass species were found to contain the long-chain n-fatty acids (Khotimchenko, 1993). Microscopic observation revealed that the SR72 sediment was composed mainly of diatomaceous phytoplankton. Diatom is probably the best candidate for the source of the long-chain n-fatty acids in the SR72 sediment. In pelagic sediments, siliceous matter is one of the important components as well as calcareous matter and red clays. Siliceous sediments as opal are known to be widely distributed around the perimeter of the Antarctic continent, along the equatorial belt in the Pacific, off the coast of Africa in the Atlantic, and in the northernmost Pacific (Broecker and Peng, 1982). Diatom is suggested to contribute to the high primary productivity in these regions (Broecker and Peng, 1982). Long-chain n-fatty acids may be widely distributed in pelagic sediments because of widespread occurrence of siliceous sediments and activity of diatoms. In fact, long-chain fatty acids were detected in the tropical Pacific Ocean (Prahla *et al.*, 1989), and in siliceous, calcareous and red clay sediments from -15°S to 50°N along 175°E in the Pacific (Ohkouchi, personal communication).

CONCLUSIONS

This study suggests that n-fatty acids ranging from C_{20} to C_{30} in marine sediments cannot be used as a reliable indicator of terrestrial organic matter input to the ocean, and that the use of biomarker approach should be accompanied with stable carbon isotopic compositions. Stable carbon isotopic compositions of individual fatty acids in sediments could give quite useful information on their terrestrial or marine sources.

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