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Article

Vertical carbon isotope change in acetate in a surface sediment from the northwestern Pacific Ocean

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Abstract

There are few carbon isotope measurements of acetate in marine sediments, even though acetate could play important roles in biological activity as well as in the carbon cycle in marine sediments. In this study, abundance and carbon isotopic composition of water-soluble acetate are investigated using a surface sediment core (ca. 30 cm) from the northwestern Pacific. A large ^{13}C-depletion in acetate by up to 14‰ is observed with increasing depth, as the concentration of acetate decreases. The depletion could reflect a kinetic isotope effect during decarboxylation of pyruvate as the sediment becomes anoxic. On the other hand, there is no difference in ^{13}C between acetate and total organic carbon (ca. –20.6‰) in the upper 13 cm of the core, which suggests that the acetate could be produced by fermentative degradation of organic matter and quantitatively consumed. The carbon isotopic composition of acetate can be useful for better understanding the carbon cycle in marine sediments.

Keywords: acetate, carbon isotopic composition, marine surface sediment, early diagenesis, microbial activity

1. Introduction

Acetate is a key intermediate for metabolic pathways and biosynthesis in most organisms. For both aerobic and anaerobic heterotrophs organic substrates are degraded to pyruvate via reductive steps, followed by decarboxylation to acetate (acetyl-CoA). Part of the acetate produced is further oxidized in the tricarboxylic acid (TCA) cycle, which discriminates respiration from fermentation. Another part of the acetate is utilized for biosynthesis. In spite of various carbon fixation pathways by autotrophic microorganisms, acetate is sufficiently abundant to be synthesized and utilized for biomass. Thus, organisms as well as the ecosystem would comprise a large acetate pool.

In previous studies, acetate in the pore water of marine sediments usually occurs at low concentration (2 to 100 μM; e.g. Blair et al., 1987; Heuer et al., 2009), probably as a result of the high demand for it from organisms as a metabolic substrate. In incubation experiments with surface marine sediments, acetate concentration increases up to 15 mM, which is interpreted as microbial activity (heterotrophic and autotrophic acetogenesis) producing a considerable amount of acetate (Wellsbury et al., 1997; Parkes et al., 2007). Thus, acetate is not only consumed as a competitive substrate for prokaryotes such as sulfate-reducing bacteria (SRB) and aceticlastic methanogens in anaerobic ecosystems (Gelwicks et al., 1994; Londry and Des Marais, 2003), but is also produced chemoautotrophically by acetogenic bacteria in marine environments (Hoehler et al., 1999, Gelwicks et al., 1989).

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To discriminate these complex acetate consumption/production processes, the carbon isotopic composition ($\delta^{13}C$) of acetate has been used. In a culture experiment, SRB grown heterotrophically consume acetate without any isotope fractionation, while the preferential uptake of ^{12}C is observed in acetate fermentation during methanogenesis, resulting in the enrichment of ^{13}C for the residual acetate by ~12‰ when 50% of the substrate is consumed (Gelwicks et al., 1994). A large isotopic effect (−58.6‰) is observed during autotrophic acetogenesis using CO_2/H_2, suggesting that the acetogen activity would reduce the ^{13}C of pore water acetate in anaerobic ecosystems (Gelwicks et al., 1989; Heuer et al., 2006).

Previous studies on the isotopic composition of acetate in marine sediment have been targeted in pore-water acetate (e.g. Blair et al., 1978; Blair and Carter Jr, 1992; Heuer et al, 2009). On the other hand, Vieth et al. (2008) suggest that a large acetate pool sorbed to the coal matrix potentially serves as a carbon source for the terrestrial deep biosphere. In marine sediment, a large acetate pool is also expected to exist behind the pore-water acetate. To our knowledge, however, a study focusing on the acetate pool in marine sediments has not been reported. In this study, we investigate cm-scale variation of the abundance and $\delta^{13}C$ values of water-soluble acetate in a surface marine sediment to provide a better understanding of microbial activity as well as of the carbon cycle during early diagenesis in marine sediments.

2. Samples and methods

2.1. Samples and bulk analyses

A surface sediment core was collected from the open marine environment of the northwestern Pacific by the R/V Hakuhomaru during expedition KH94-3 (September to October, 1994) at site LM-8 (38°53.2´N; 143°22.3´E), using a multiple corer (Fig. 1). The site is on the warm Kuroshio Current, and the water depth is 2349 m. The sediment was grey mud without significant bioturbation. An oxidized layer was identified in most surface sediment (0-2 cm, Yamane and Oba, 1999), suggesting oxygen penetration into this depth. The sediment core in a core-liner was immediately stored in a freezer after core retrieval, followed by subsampling every 1 cm on board the ship. The sediment core samples were preserved at −20ºC before analysis and those collected from eight different horizons (0-1, 1-2, 2-3, 3-4, 6-7, 12-13, 20-21 and 28-29 cm) were used in this study. Chemical components such as concentrations of pore-water sulfate, alkalinity and methane had not been analyzed at the time of core retrieval. Although these data are important to constrain *in situ* microbial activities, those chemical properties are not analyzed in this study because they can be altered from their original components. Any biogeochemical alteration in concentration and isotopic composition of acetate, however, should not occur during the preservation.

The samples were freeze-dried under reduced pressure, followed by homogenization. The powdered samples were treated with 6M HCl to remove carbonate carbon, and then dried under reduced pressure. TOC content and its $\delta^{13}C$ value were determined using an elemental analyzer (EA, FISON NA1500) and a Finnigan delta S isotope ratio mass spectrometer interfaced to the EA. The analytical precision for the standards was less than 0.2‰.

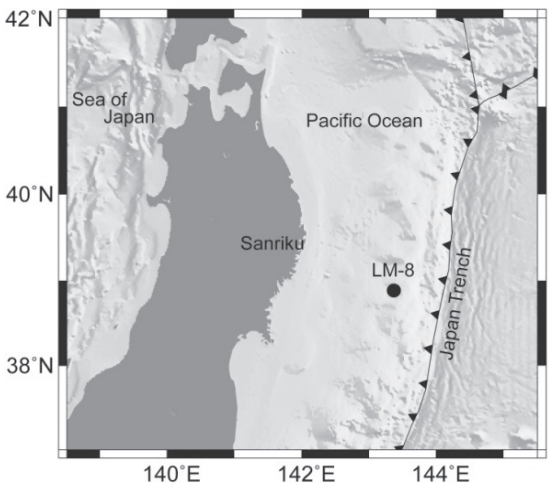

Fig 1. Location of multiple coring in Pacific Ocean off Sanriku, Japan.

2.2. Acetate analysis

Extraction and carbon isotope analyses of acetate were carried out using a method modified after Oba and Naraoka (2006). The extraction was conducted in closed system to prevent from contamination. Oba and Naraoka (2006) analyzed carbon isotopic composition of acetate using gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS). We also employed this method to analyze the isotopic composition with existent facilities, although recent studies have developed liquid chromatography (LC)/IRMS for the analysis of volatile fatty acids (Krummen et al., 2004; Heuer et al., 2006). The GC/C/IRMS has advantages in the followings: shorter analysis time, smaller sample volume (20 ngC) and less daily maintenance such as a buffer preparation, and cleaning of pump heads, compared to those of LC/IRMS.

Acetate was extracted with distilled and deionized water (hereafter termed simply water). A schematic figure of an experimental procedure is shown in Fig. 2A. Briefly, the freeze dried sediment (100-150 mg) was ultrasonically extracted with water (ca. 3 ml), assisted by 50 freeze-thaw cycles in a degassed and sealed Pyrex tube at natural pH (ca. pH 8.2). The supernatant was separated by centrifugation and the residual sediment was further washed with the distilled water (1 ml, 3 x). The combined solution was freeze-dried. After the resulting residue was re-dissolved in 300-400 μl water, the solution was decanted to a section of a two-way glass tube (Fig. 2.B). Ca. 200 μl of 85% H_3PO_4 was added to another section of the tube. The tube was then evacuated and sealed under reduced pressure. The solution was mixed to convert acetate to acetic acid. The acidified solution was distilled at ca. 250℃ and trapped in another section of the tube at liquid N_2 temperature. As the acetic acid standard was added to organic-free sea sand and extracted with alkaline water and recovered as above, this extraction procedure achieved >90% recovery. In addition, the marine sediment (3-4 cm) was analyzed in duplicate to check the reproducibility and efficiency of the extraction.

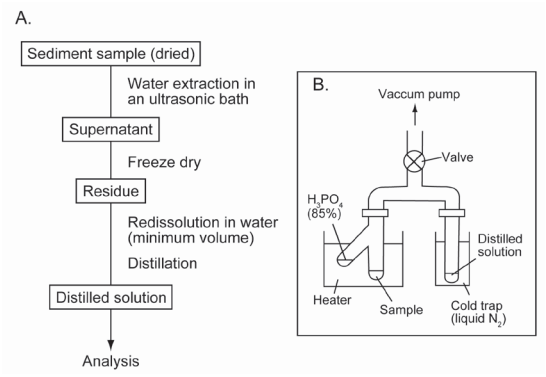

Fig 2. A., A schematic figure for an experimental procedure: B., a scheme of a distillation system.

The distilled solution was analyzed using gas chromatography (GC) with a Hewlett-Packard 5890 II gas chromatograph equipped with a flame ionization detector by splitless injection using a DB-FFAP column (30 m × 0.32 mm i.d., 0.25 μm film thickness). The GC oven temperature was held at 40℃ for 2 min, heated to 100℃ at 20℃/min, then to 200℃ at 2℃ /min, and held constant for 5 min. Quantification was conducted by comparing the peak area with a standard solution of acetic acid. Acetate concentration is given as μmol/g dry sediment (hereafter μmol/g). Carbon isotopic composition was determined using a GC/combustion/isotope ratio mass spectrometer (Finnigan delta S) interfaced with a Hewlett-Packard 5890 II gas chromatograph with a DB-FFAP column (60 m × 0.32 mm i.d., 0.25 μm film thickness). The micro volume combustion furnace was operated at 940℃ with CuO and Pt wire as oxidant and catalyst, respectively. Samples were injected using splitless mode at 200℃ and the GC oven was held at 40℃ for 2 min, heated to 80℃ at 20℃/min, then to 130℃ at 2℃/min, and to 200℃ at 20℃/min. The standard deviation during two or three injections was <1.6‰. No substantial isotopic fractionation (<1‰) was observed when the recovery of the extraction was high (> 90%; Oba and Naraoka, 2006).

3. Results and discussion

3.1. TOC content and δ^{13}C value of TOC

TOC content ranges from 1.3 to 2.0 wt%, increasing at ca. 10 cm depth (Table 1). On the other hand,

the $\delta^{13}C$ values ($\delta^{13}C_{TOC}$) are relatively constant (ave. −20.6 ± 0.2‰) over the ca. 30 cm core (Table 1). This isotopic signature indicates that sedimentary organic matter derived from mainly marine origin (Naraoka and Ishiwateri, 2000). In general, the degradation of organic matter (OM) in marine sediments preferentially involves the metabolizable OM; however, the isotopic change in $\delta^{13}C_{TOC}$ is small because of the small fraction of metabolizable OM relative to refractory OM (Blair and Carter Jr, 1992; Meyers, 1997). The constant $\delta^{13}C_{TOC}$ (ca. −20.6‰) suggests that the origins of the bulk OM are very similar or constant through the entire core, being primarily derived from marine algae.

3.2. Acetate distribution

Acetic and propionic acid were identified in the sediment samples (Fig. 3). Duplicate analysis of the sediment (3-4 cm) gives a consistent concentration (7.3 and 7.4 μmol/g), representing high reproducibility. In addition, when the residue after extraction was further extracted with 2 ml of water using the same method, no acetate was detected (i.e. below detection limit). These results indicate that ca. 100% of the acetate is recovered in this study. As bioturbation or animal tissue is not apparent in the core, the acetate could originate from microbial activity and clay-absorbed OM (see below).

The concentration of acetate maximizes at 9.3 μmol/g in the uppermost sediment (0-1 cm) and decreases from 8.3 to 1.4 μmol/g below 2-3 cm (Fig. 4). The observed acetate concentration is higher (up to 1000 x) than the values reported for pore-water acetate (<100 μM) in marine sediments (e.g. Blair et al., 1987, 1992; Heuer et al., 2006) but lower than that in incubated sediment (ca.15 mM; Wellsbury et al., 1997; Parkes et al., 2007). These results suggest that a large pool of acetate exists in marine surface sediments rather than in pore-water. The bacterial population is likely to be an important acetate source, as the ultrasonic extraction employed here destroys microbial cells. Acetate adsorbed to clay minerals is also likely to be extracted using sonication (Vieth et al., 2008). Any acidic and basic solution was not added during the water extraction, hence, acetate extracted in this study should not include one chemisorbed to macromolecular organic matter. Such water soluble acetate is available as a carbon substrate for ecosystem in the marine sediment.

3.3. $\delta^{13}C$ change in acetate

The $\delta^{13}C$ value of acetate in the sediments is relatively constant (ca. −20.6‰) above 12-13 cm, whereas acetate becomes depleted by 4.5‰ at 20-21 cm and 9.7‰ at 28-29 cm (Fig. 4). Such drastic ^{13}C-depletion with depth could be caused by (i) variation in $\delta^{13}C$ value of source OM, (ii) a significant contribution of ^{13}C-depleted acetate produced by litho-autotrophic acetogens, and/or (iii) change in the degree of isotopic fractionation during fermentation of OM to

Table 1. Content and carbon isotopic compositions of TOC and acetatea* in sediments from the Pacific Ocean off Sanriku, Japan.

Sediment Depth	Bulk		Acetate		
	TOC (wt%)	$\delta^{13}C$ (‰)	[μmol/g]	$\delta^{13}C$ (‰, in ave.)	Std. dev.
0-1cm			9.3	–21.3	0.3
1-2 cm	1.3	–20.9	2.5	–21.8	0.7
2-3 cm			8.3	–19.4	0.2
3-4 cm			7.3	–19.9	0.4
6-7 cm	1.3	–20.5	3.8	–21.1	1.3
12-13 cm	1.9	–20.7	2.4	–20.3	1.6
20-21 cm			1.3	–24.8	0.6
28-29 cm	2.0	–20.5	1.4	–34.5	1.1

*Averaged isotopic compositions and standard deviations (Std. dev) from duplicate or triplicate injections.

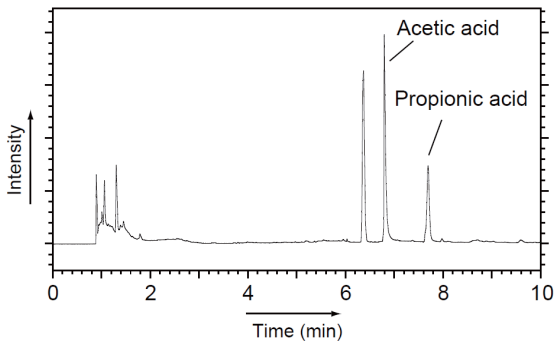

Fig. 3. Partial gas chromatogram extracted from sediment core (3-4 cm). 1 = acetic acid, 2 = propionic acid.

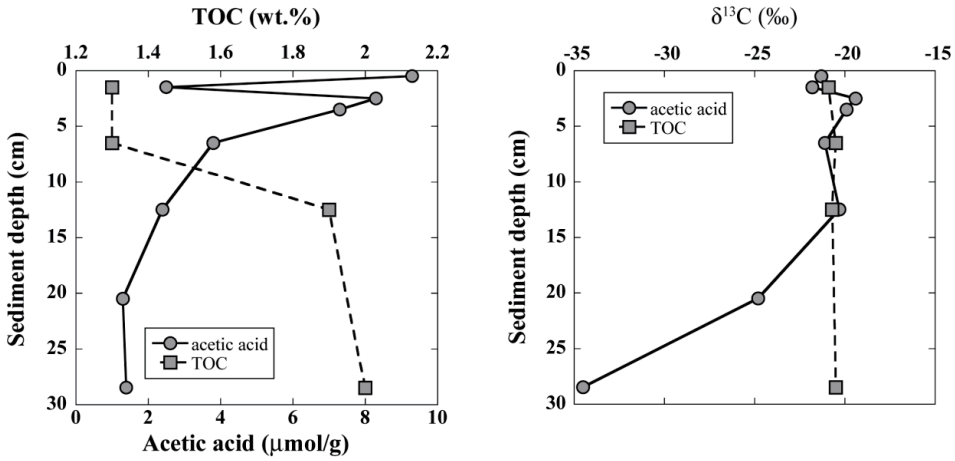

Fig 4. Depth profiles of concentration and $\delta^{13}C$ of acetic acid and TOC.

acetyl-CoA.

The ^{13}C-depletion of acetate should not reflect a change in $\delta^{13}C$ value of organic sources because $\delta^{13}C_{TOC}$ is constant within the entire core. On the other hand, Acetogens produce acetate with low $\delta^{13}C$ value (−58.6‰, Gelwicks et al., 1989), which could reduce the $\delta^{13}C$ value of pore-water acetate (Gelwicks et al., 1989; Heuer et al., 2006). However, in open-marine surface sediments, the acetogenic activity seems to be inhibited in the sulfate reduction zone because SRB outcompete lithoautotrophic acetogens for H_2 as a competitive substrate (Hoehler et al., 1999). Although the sulfate concentration was not measured in this study, its consumption within 30 cm depth seems difficult. Usually the complete sulfate consumption is observed below 10 m depth for open marine sediments (e.g. Zierenberg and Holland, 2004).

Lever et al. (2010) suggest that although acetogenesis has a disadvantage in thermodynamics compared with sulfate reduction, non-lithoautotrophic acetogens such as organoauto- and organoheterotrophic acetogens can survive with higher metabolic versatility, especially, capability of dimethylating lignin monomers in marine sediments with high terrestrial organic matter contribution. Because a reductive acetyl-CoA pathway utilized by these acetogens for carbon assimilation involves a large isotope fractionation, their contribution could explain ^{13}C-depleted acetate production. On the other hand, the terrestrial contribution is insignificant in the sediment used in this study (Table 1 and Naraoka and Ishiwateri, 2000), hence the concentration of lignin monomers derived from vascular plants should be much lower than that of Lever et al. (2010). It is ambiguous if the non-lithoautotrophic acetogens can survive in this circumstance and if acetate from the acetogens can account for the $\delta^{13}C$ variation of such a large acetate pool. When it is proved that microbes with the reductive acetyl-CoA pathway have a large population, it can explain the ^{13}C-depletion of acetate. However it is beyond of the scope of this study.

In contrast to the carbon assimilation by the reductive acetyl-CoA pathway, an organic matter dissemination pathway of glycolysis is employed by most of organisms and a wide variety of carbon substrates are degraded to acetate via pyruvate (Nelson and Cox, 2000). DeNiro and Epstein (1977) reported the isotope fractionation with pyruvate decarboxylase of yeast to conclude that the extent of fractionation during decarboxylation of pyruvate is inversely proportion to the amount of available pyruvate converted to acetyl-CoA. This kinetic isotope fractionation does not occur when pyruvate is completely converted to acetyl-CoA. The similar isotopic composition between acetate and TOC above 13 cm depth is likely to be produced by effective

oxidation of pyruvate with little isotopic fractionation. Furthermore, despite the decrease in acetate concentration from 9.3 to 2.4 μmol/g with depth, the isotopic composition is constant (ca. −20.6‰). This uniform value also suggests consumption of acetate without significant isotopic fractionation by microbes such as sulfate reducing bacteria (Londry and Des Marais, 2003). The acetate consumption without the isotopic consumption can continue to occur below 13 cm, because sulfate reduction zone would be expanded to 30 cm or deeper. On the other hand, as the redox conditions shift to anoxic with increasing depth, fermentation of OM to pyruvate (a reductive step) results in an increase in the pyruvate pool, while the extent of decarboxylation of pyruvate to acetyl-CoA (an oxidative step) decreases to promote an extensive kinetic isotope fractionation. In fact, the decomposition rate of organic substrate to pyruvate increases ten times in an anoxic environment (Nelson and Cox, 2000). In addition, as the TCA cycle is inhibited in an anoxic environment, acetate formation could be depressed. The decrease of acetate concentration with depth is consistent with this process under the anoxic condition. The isotope fractionation with the pyruvate decarboxylase of yeast (ca. 9‰) was observed when 8% of the pyruvate was converted to acetate at 35℃ (DeNiro and Epstein, 1977). Probably, the isotopic fractionation would be greater with the anaerobic processes at the lower temperature (ca. 3℃) in deep-sea sediment. Therefore, the occurrence of acetate with up to 15‰ fractionation in this study is likely to be caused by change in microbial metabolisms from aerobic to anaerobic activities. The transition of aerobic/anaerobic metabolic pathways could be within 13-20 cm depth.

4. Concluding remarks

Acetate extracted from the marine surface sediment using ultrasonic extraction with water is more abundant than reported for acetate in pore-water, indicating the presence of a large pool of acetate in sediment. In the upper part of the sediment, the δ^{13}C value of acetate (ca. −20.6‰) is similar to that of TOC. In this region, organic substrate is quantitatively decomposed to acetate, resulting in increase of acetate concentration in this depth. Although, acetate consumption is also expected, insignificant isotopic fractionation should be involved during this process. On the other hand, in the deeper sediment down to ca. 30 cm, the δ^{13}C value of acetate decreases with depth from −20.3‰ to −34.5‰. Such a dramatic δ^{13}C-depletion could be responsible for fermentative degradation of OM in anaerobic environment, which causes a large isotopic fractionation during decarboxylation of pyruvate. The transition of aerobic/anaerobic metabolic pathways would be within 13-20 cm. Further studies on not only the isotopic composition of acetate (including the intramolecular isotopic composition), but also on the isotopic fractionation between acetate and biomass in culture under *in situ* conditions are required to estimate the precise isotopic effect during these biogeochemical processes. Pore-water chemistry and microbiology of sediments should be also incorporated to constrain the acetate sources and microbial activities.

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