Natural Abundances of Carbon Isotopes in Acetate from a Coastal Marine Sediment

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Measurements of the natural abundances of carbon isotopes were made in acetate samples isolated from the anoxic marine sediment of Cape Lookout Bight, North Carolina. The typical value of the total acetate carbon isotope ratio ($\delta^{13}C$) was -16.1 ± 0.2 per mil. The methyl and carboxyl groups were determined to be -26.4 ± 0.3 and -6.0 ± 0.3 per mil, respectively, for one sample. The isotopic composition of the acetate is thought to have resulted from isotopic discriminations that occurred during the cycling of that molecule. Measurements of this type, which have not been made previously in the natural environment, may provide information about the dominant microbial pathways in anoxic sediments as well as the processes that influence the carbon isotopic composition of biogenic methane from many sources.

THE NATURAL ABUNDANCES OF CARBON isotopes in acetate and its methyl group have been determined for samples isolated from an anoxic coastal sediment. To our knowledge, an intramolecular isotopic measurement has not been reported previously for a compound recovered from the natural environment. The methyl group of the acetate molecule was significantly enriched in ^{12}C relative to the carboxyl group. This finding suggests that isotopic fractionations of carbon occurred during the microbial turnover of acetate. These measurements can provide new information on the biogeochemical cycling of carbon in anoxic environments and represent a novel tracer approach for the study of the early diagenesis of organic matter.

Acetate occupies a central role in the degradation of organic matter in anoxic systems. Produced by acid-forming bacteria, for example, *Clostridia* or *Acetobacter* (*1*), acetate is a substrate for methanogenesis in freshwater lake and marine sediments, rice paddies, and sewage digesters (*2*), as well as for the major portion of sulfate reduction in the marine environment (*3*). Its significance to the biogeochemical cycling of carbon is amplified when one considers that biogenic sources of methane, such as rice paddies, wetlands, and cattle, are thought to be the most important contributors to the atmospheric pool and are possibly responsible for increases in tropospheric methane concentrations (*4*). In principle, global methane budgets that describe the relative contributions of different sources may be constrained by mass balance calculations that use isotopic compositions (^{12}C, ^{13}C, ^{14}C, ^{1}H, ^{2}H) and atmospheric fluxes of methane from the major sources (*5*). Studies of the in situ methanogenic pathways, which include acetate fermentation, will be required to understand the processes that control these parameters. Isotopic fractionations in acetate cycling have been invoked to explain the $^{13}C/^{12}C$ composition observed in methane from various sources (*6*), but, to our knowledge, no isotopic measurements of acetate from the natural environment have been made to test that hypothesis.

Our measurements were made on acetate isolated from the anoxic sediments of Cape Lookout Bight, North Carolina. Recently, seasonal variations in the $^{13}C/^{12}C$ content of methane were measured there (*7*). If these variations prove to be a general phenomenon, then undersampling in studies designed to determine isotopic signatures of methane sources would be a problem. The seasonal variations have been attributed in part to changes in the methanogenic pathways which include acetate cycling.

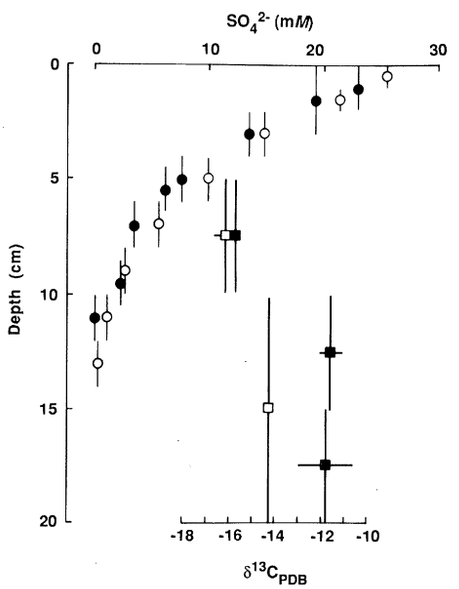

Fig. 1. Acetate $\delta^{13}C$ values (*16*) in samples isolated from Cape Lookout Bight sediments. Samples were collected on 6 July (□), and 7 July 1983 (■). Each point represents the average of two determinations (indicated by the end points of the bars) with the exception of the point for the depth interval of 15 to 20 cm (7 July). The horizontal bar for that point includes the estimated error associated with the correction of an analytical isotope effect (see text). Sulfate profiles (determined by ion chromatography) are shown for samples collected 20 June (●) and 20 July 1983 (○). Significant methane production from acetate begins at depths below which the sulfate concentration is depleted to less than 2 m*M* (*12*).

Cape Lookout Bight is an organic-rich coastal basin about 2 km^2 in area located 115 km southwest of Cape Hatteras on the North Carolina Outer Banks (*8*). The high sedimentation rate measured for the upper meter of sediment, 10.3 ± 1.7 cm $year^{-1}$ (*9*), supports a large burial rate for organic matter and as a consequence high rates of decomposition of organic matter (*8*). During summer months, sulfate reduction occurred at depths between 0 and 10 cm (*8*). Methanogenesis occurred at measurable rates at depths between 10 and 40 cm (*10*). Acetate concentrations over 100 μ*M* were found in association with these rates (*11*). Primary sinks of acetate included oxidation by sulfate-reducing bacteria (*11*) and fermentation by methane-producing bacteria (methanogens) (*12*). Approximately 20 to 30% of the methane was derived from the acetate fermentation pathway, the remainder from CO_2 reduction. The acetate fermentation and the sulfate reduction zones did not overlap (*11, 12*) because the sulfate reducers can apparently outcompete the methanogens for the acetate (*13*).

We obtained interstitial water samples of about 100 ml by squeezing diver-collected cores with a sediment press (*14*). Our first samples were obtained on 6 and 7 July 1983. Interstitial water from five cores 5 cm in diameter was pooled to obtain sufficient sample volume at each depth in the sediment column. For the sample taken on 13 June 1984, sufficient interstitial water was obtained from a single core 9.5 cm in diameter. All samples were kept frozen until analysis. The interstitial water samples were acidified with H_3PO_4 to *p*H 1 and cryogenically distilled in vacuo. The *p*H of the distillates was increased to 12 by adding 2.7*N* NaOH. The distillates were then dried at 100°C. The isotopic analysis of the acetate proceeded as previously reported (*15*), except that all solutions were adjusted to *p*H 12 instead of *p*H 10 to 11 before drying at 100°C. A comparison was made of the $\delta^{13}C$ values (*16*) of a sodium acetate standard before and after processing. Negligible fractionation was observed for standards with concentra-

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tions ≥150 μ*M* (*17*). All but one of the samples had concentrations ≥150 μ*M*. The typical precision (SD) of the measurement was ±0.3 per mil ($n = 7$). A significant fractionation was seen for standards with concentrations <100 μ*M*. The sample collected on 7 July 1983 from the sediment depth interval of 15 to 20 cm had a concentration of 80 μ*M*; the $\delta^{13}C$ value was corrected by -3 ± 1 per mil by using a standard curve. The sample-size requirement limits the application of this procedure to environments characterized by high rates of remineralization of organic carbon because acetate concentrations in most sediments are typically less than 100 μ*M* (*18*). Recent work in our laboratory indicates that analyses can be performed with similar procedures on samples with concentrations of acetate as low as 20 μ*M* (*19*).

Pyrolysis of acetate at 500°C in excess NaOH yielded methane derived exclusively from the methyl group (*20, 21*). The methane was then combusted for the intramolecular isotopic analysis (*15, 21*). The precision (SD) of the measurement was ±0.4 per mil ($n = 14$). No significant isotope fractionation was observed for pyrolyses with yields of methane >96%. Accordingly, only those measurements that were the result of pyrolyses with yields >96% are reported.

The total acetate $\delta^{13}C$ values are shown in Fig. 1 (*16*). The $\delta^{13}C$ values of acetate isolated from the depth interval from 5 to 10 cm were -16.2 ± 0.4 per mil (mean ± SEM, $n = 2$) and -15.8 ± 0.2 per mil for the collection dates 6 and 7 July 1983. The isotopic composition of the acetate from depths of 10 to 20 cm ranged from -14.4 ± 0.1 to -11.6 ± 0.4 per mil ($n = 2$ for each) for the same respective dates. The $\delta^{13}C$ value of the total organic carbon (TOC) fraction ranged from −19.3 per mil ($n = 1$) for the depth interval from 6 to 9 cm to −18.4 per mil ($n = 1$) at a depth of 12 to 15 cm for a core collected on 7 July 1983. The TOC $\delta^{13}C$ value gradually returned to −19.3 per mil ($n = 1$) at a depth of 36 to 40 cm to yield a mean $\delta^{13}C$ value for the entire core of -19.0 ± 0.3 (mean ± SD, $n = 11$).

Intramolecular $\delta^{13}C$ measurements of acetate were made on samples collected on 13 June 1984 that consisted of interstitial water from the depth interval of 8 to 16 cm. The value for the methyl group was -26.4 ± 0.3 per mil ($n = 2$) for acetate whose total $\delta^{13}C$ value was -16.2 ± 0.1 per mil ($n = 2$). The value for the carboxyl group was determined by a mass-balance calculation to be -6.0 ± 0.3 per mil. The estimated difference between the values for the methyl and carboxyl groups is 20.4 ± 0.4 per mil. Because these measurements are from a single core, it is premature to conclude that this value is typical of Cape Lookout Bight sediments. However, the $\delta^{13}C$ value of the total acetate from the same sample appears to be typical of the Cape Lookout environment.

The mean $\delta^{13}C$ value of the acetate samples (-14.3 ± 2.1, $n = 6$) is significantly different from the mean $\delta^{13}C$ value of the TOC fraction (-19.0 ± 0.3, $n = 11$). This observation, along with the difference (20 per mil) between the methyl and carboxyl groups in one core, suggests that substantial isotope fractionation has occurred during the cycling of acetate. This is expected; the synthetic (*1*) and degradative (*22*) pathways identified as operating in anoxic environments have the potential to discriminate between carbon isotopes to varying degrees (*23*). Our calculated difference between the methyl and carboxyl groups is within the range of values reported for biologically synthesized acetate (3 to 24 per mil) (*15, 21, 24*). The ^{13}C depletion of the methyl group relative to the carboxyl group was typical in those studies. The intramolecular difference (24 per mil), which was most similar to our findings, was produced by an aerobic culture of *Escherichia coli* grown on glucose (*15*). The fractionation was hypothesized to result from the interconversion of acetyl phosphate and acetyl coenzyme A (*15*). Additional studies are required to determine if distinct isotopic signatures can be correlated with different metabolic pathways that involve acetate. The interpretation of an isotopic signature will be difficult in complex natural systems; however, multiple sources of fractionation might be decoupled with the use of selective inhibitors for specific microbial pathways, such as sulfate reduction (*3*). Acetate and other key microbial substrates have different degradative pathways in sulfate-reducing, methane-producing, and other biogeochemical zones. Determination of intramolecular carbon isotope variations as a function of zone would allow the comparison of different microbial pathways within the same sediment. If we can understand the isotopic systematics of acetate cycling, this type of measurement could prove to be a sensitive monitor of changes within a microbial ecosystem as a function of time, depth in sediment, or temperature.

Variations in the relative importance of acetate-fermentation and CO_2-reduction pathways have been proposed to explain isotopic differences between methane produced in freshwater and marine environments (*25*) as well as seasonal $\delta^{13}C$ variations in methane at Cape Lookout (*7*). Measurements of the $\delta^{13}C$ values of acetate and dissolved inorganic carbon (DIC) along with ^{14}C-tracer rate studies will allow these hypotheses to be tested. It is our intention to use this approach to develop a model that describes in quantitative terms the microbial processes that control the isotopic composition of methane. At this time we cannot extrapolate our findings to other systems, given the limited data set. Even so, a large intramolecular heterogeneity in acetate, similar to that seen at Cape Lookout, has been invoked to explain the isotopic signatures of methane and DIC from other environments such as freshwater systems (*6*). If actual measurements prove that to be the case, it would suggest that the processes that control the $\delta^{13}C$ values of acetate and methane are remarkably similar for the different environments.

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16. Values of $\delta^{13}C$ (per mil) relative to that for the Pee Dee belemnite carbonate (PDB) standard are defined by the equation: $\delta^{13}C_{PDB}$ (per mil) = $[R_{sample}/R_{PDB}) - 1] \times 1000$, where $R_{sample} = {}^{13}C/{}^{12}C$ and $R_{PDB} = 0.0112372$.
17. δ (before processing) − δ (after processing) = -0.6 ± 0.5 per mil ($\bar{x} \pm$ SD, $n = 7$). The unprocessed standard was prepared with the bomb combustion technique as described previously (*15*) except that quartz boats and silver wire were used in place of silver boats. Interlaboratory calibration of the bomb combustion technique was accomplished with use of the National Bureau of Standards 22 standard (*15*). This technique was also used to combust acidified sediments for the TOC analysis.
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Antiarthritic Gold Compounds Effectively Quench Electronically Excited Singlet Oxygen

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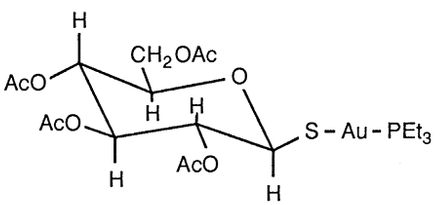
Although certain gold [Au(I)] compounds have been used effectively in the treatment of rheumatoid arthritis for some years, the molecular basis for such therapeutic action has been unclear. One possible mechanism of the action of Au(I) compounds is that they protect unsaturated membrane lipids and proteins against oxidative degradation caused by activated phagocytes that are not properly regulated. In this study it has been shown that superoxide ion ($O_2^{\cdot-}$), a product of activated phagocytes, can be oxidized to electronically excited singlet oxygen ($O_2^1\Delta_g$), an agent that is capable of peroxidation of unsaturated fatty acid derivatives. It has also been shown that antiarthritic Au(I) compounds are effective deactivators of $O_2^1\Delta_g$ with quenching constants on the order of $10^7\ M^{-1}\ sec^{-1}$.

A MAJORITY OF PATIENTS WITH rheumatoid arthritis (RA) who do not respond to nonsteroidal anti-inflammatory drugs benefit from a course of therapy with certain compounds of Au(I), for example, tetra-*O*-acetylglucose-1-thiol gold(I) triethylphosphine complex (auranofin; Ac is acetyl group and Et is ethyl group)



Auranofin

(*1, 2*). Such treatment is more effective in the early rather than the later stages of RA before extensive destruction of joint bone and tissue occurs; it also appears to retard further erosion. The molecular basis of the antiarthritic activity of Au(I) compounds remains unclear, although several possibilities have been discussed that include: (i) inhibition of thiol-dependent proteases such as cathepsins (*3*); (ii) inhibition of cellular function of various phagocytes, for example, chemotaxis of neutrophils (*4, 5*) and superoxide ion ($O_2^{\cdot-}$) (*6*) or oxyradical generation (*7*); (iii) prevention of myeloperoxidase-induced inactivation of α-1-proteinase inhibitor (*8*); (iv) inhibition of release of lysosomal enzymes (*9*); and (v) inhibition of adenosine diphosphate or collagen-induced platelet aggregation (*10*). Most of the current research on RA is guided by the idea that the disease is associated with deficiencies in regulation of the immune response that result in excessive inflammation and irreversible deterioration of joints. Lipid peroxidation and subsequent free radical–promoted degradation of protein (which includes protective proteins such as superoxide dismutase and α-1-antiproteinase, as well as proteins of cartilage and ligament) are thought to be important degenerative processes (*11*). In this report we show that Au(I) compounds might inhibit the peroxidation of membrane lipids.

Superoxide ion, which is produced in abundance by activated phagocytes such as neutrophils and macrophages, has been shown to effect biodegradation (*12*). Initial proposals that this and other toxic effects may result from the formation of hydroxyl radicals from superoxide ion have not received support (*13*), and leave open the question of how the relatively innocuous superoxide ion (*14–16*) could effect lipid peroxidation and other oxidative damage. The view that superoxide ion can be deleterious in biological systems derives support from the existence of superoxide dismutase (SOD) proteins that catalyze the conversion of $O_2^{\cdot-}$ to H_2O_2 and O_2 (ground state) at a rate ($k = \sim 2 \times 10^9\ M^{-1}\ sec^{-1}$), which is even greater than that for the rapid spontaneous second-order process in neutral water ($k = 10^7\ M^{-1}\ sec^{-1}$) (*17, 18*). SOD also displays anti-inflammatory activity. A simple reason for the protective function for SOD could be the existence of another pathway for toxicity (independent of H_2O_2 or $HO\cdot$), specifically, the direct oxidative conversion to electronically excited singlet oxygen ($O_2^1\Delta_g$). We have demonstrated this conversion unambiguously in a simple chemical experiment. Addition of a solution of potassium superoxide in acetonitrile that contains 18-crown-6 ether (*15, 16*) to a solution of excess cerium(IV) ammonium nitrate in acetonitrile rapidly produced singlet oxygen, as shown by the measurement of intense and characteristic emission at 1270 nm with the detection system previously described (*19*) (see Fig. 1) (*20, 21*). In addition, the emission spectrum corresponds to that characteristic of singlet oxygen (*19*). The formation of $O_2^1\Delta_g$ from $O_2^{\cdot-}$ also occurs with other oxidants, for example, with lead tetraacetate, iodobenzene diacetate, and tetranitromethane in acetonitrile. Oxidation of $O_2^{\cdot-}$ to $O_2^1\Delta_g$ within or near the surface of biological membranes, for example, by cytochrome oxidases, is therefore a reasonable possibility. The peroxidation of unsaturated fatty acids by singlet oxygen and the in vivo toxicity of photochemically generated singlet oxygen are both well established (*22*).

In addition to showing that superoxide ion can be converted directly to singlet oxygen by oxidation, we have established a

Table 1. Rate constants for the quenching of $O_2^1\Delta_g$ by various agents according to two methods. Method A: solvent, $CFCl_2CF_2Cl$; $O_2^1\Delta_g$ generated from 1,4-dimethylnaphthalene-1,4-endoperoxide at 30°C. Method B: solvent, benzene; $O_2^1\Delta_g$ generated by self-sensitized photooxidation of rubrene at 30°C.

Quencher	$k_q\ (M^{-1}\ sec^{-1})$	
	MethodA	Method B
Auranofin	0.75×10^7	0.2×10^7
$(C_2H_5)_3PAuSCH_3$	4.5×10^7	3.7×10^7
$(C_6H_5NHCS_2)_2Ni$	7.2×10^9	4.4×10^9
β-Carotene	1.1×10^{10}	1.5×10^{10}

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