Source(s) and cycling of the nonhydrolyzable organic fraction of oceanic particles

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Abstract

A major fraction of particulate organic carbon (POC) in the deep ocean remains molecularly uncharacterized. In an effort to determine the chemical characteristics and source(s) of sinking POC, we studied a nonhydrolyzable fraction of sinking POC using 13C NMR (nuclear magnetic resonance) spectroscopy and analytical pyrolysis. 13C NMR spectra and products from analytical pyrolysis of the nonhydrolyzable fraction exhibit a strongly aliphatic character that is distinct from that of bulk POC. The aliphatic nature of this fraction is consistent with its low stable carbon isotope values. We hypothesize that the nonhydrolyzable fraction derives to a significant extent from a refractory component of organisms that selectively accumulates, resulting in its manifestation as a major part of POC sinking to the deep ocean and in underlying sediments.

1. Introduction

Understanding the fluxes, transformations, and fate of particulate organic carbon (POC) is a major focus of oceanic carbon cycle research. In particular, how and what fraction of POC evades remineralization in the water column is a subject of intense study. Less than half of sinking POC and sedimentary organic carbon (SOC) has been molecularly characterized (Wakeham et al., 1997), and the molecularly uncharacterized component [MUC, (Hedges et al., 2000; Lee et al., 2004)] accounts for an increasing fraction of sinking POC with increasing water depth. A major reason why the majority of POC remains uncharacterized is because it can neither be extracted with organic solvents nor be hydrolyzed with strong non-oxidizing acids or bases for further analysis. In addition, only a fraction of the extracted or hydrolyzed organic matter can be characterized by chromatographic and/or mass spectrometric analyses. There have been attempts to characterize sinking POC using other approaches. 13C NMR (nuclear magnetic resonance) spectroscopy helps identify carbon structure and functional groups of POC, and hence relative contributions of compound classes such as lipids, carbohydrates, and protein (Hedges et al., 2001). Comparison between 13C NMR and molecular analysis results indirectly implies that MUC has a similar chemical composition to the corresponding bulk sinking POC. Hedges et al. (2001) studied 13C NMR spectra of plankton, and sinking POC at both shallow and deep ocean depths in the Equatorial Pacific and the Arabian Sea. These analyses yielded similar spectra with no obvious enhancement of signal for any specific chemical components at depth, implying nonselective preservation of POC throughout the water column.

Another approach is to study a nonhydrolyzable fraction of sinking POC. The nonhydrolyzable fraction is organic carbon that remains after extraction with organic solvents and hydrolysis with a strong non-oxidizing acid. This fraction is not directly comparable to MUC, because MUC contains other hydrolyzable or solvent-soluble, but yet uncharacterizable components in addition to the nonhydrolyzable fraction. Nevertheless, the nonhydrolyzable
fraction accounts for up to 50% of sinking POC in the deep northeast Pacific (Wang et al., 1998; Hwang and Druffel, 2003). Consequently, there must be significant overlap in organic matter pools defined as “molecularly uncharacterized” and “nonhydrolyzable.” Moreover, determining the chemical composition of the latter undoubtedly improve our understanding of the sources and transformations of POC in the ocean.

Studies of carbon isotope ratios of the organic fractions of sinking POC in the deep northeast Pacific showed that both radio- and stable-carbon isotope signatures of the nonhydrolyzable fraction were distinct from those of corresponding amino acids and carbohydrates, but similar to those of lipids (Wang et al., 1998; Hwang and Druffel, 2003). Based on these results, it was suggested that the nonhydrolyzable fraction might constitute a selectively preserved “lipid-like” component of organisms (Hwang and Druffel, 2003).

In this paper, we use solid state CP/MAS (cross polarization/magic angle spinning) $^{13}$C NMR spectroscopy and analytical pyrolysis coupled with gas chromatography/mass spectrometry (pyrolysis-GC/MS) to further characterize the nonhydrolyzable fraction of sinking POC, and evaluate whether this fraction is “lipid-like,” as prior carbon isotope results have indicated.

2. Methods

2.1. Sample collection and treatment

Sinking POC samples were collected with a conical sediment trap moored at a depth of 3450 m, 650 m above bottom at Station M in the northeastern Pacific (34°50’N, 123°00’W, 220 km west of the central California coast, USA) (Smith and Druffel, 1998). Trap sampling cups were poisoned with mercuric chloride. Because of large sample amount necessary for NMR spectroscopic analyses, fractions of eighteen 10-day period samples collected from December 1997 to December 1998 (Hwang et al., 2004) were combined and homogenized for spectroscopic and mass spectrometric analyses. A surface (0.2–0.5 cm depth horizon) sediment sample was collected at a site on the adjacent continental slope (1866 m water depth, 34°28’N, 121°19’W) using a multi-corer during the MULVFS cruise in August 2001.

A series of chemical treatments were used to isolate the nonhydrolyzable organic matter. With the exception of the bulk sinking POC sample, all samples were demineralized in order to remove interfering paramagnetic substances and improve the signal of $^{13}$C NMR spectroscopy. The method used for compound class separation by Wang et al. (1998) was adopted to remove lipids and amino acids. To prevent potential artifact formation via condensation reaction (Allard et al., 1998), carbohydrates were removed before hydrolysis. Briefly, aliquots of dried sinking POC (2.2 g) and sediment (5.4 g) were acidified with 30 ml of 6 N HCl overnight at room temperature to remove carbonate minerals. Supernatant was removed after centrifugation following each treatment. The residues were demineralized twice with 50% HF for 12 h each time at room temperature. To remove extractable lipids, samples were ultrasonicated using Tekmar Sonic Disruptor in a dichloromethane methanol 2:1 (volume:volume) mixture for 5 min and refrigerated (4 °C) overnight. Lipid extraction was then performed once more by the same method. The residues were treated twice with trifluoroacetic acid (2 N, 20 ml) for 2 h at room temperature, to remove carbohydrates (Allard et al., 1998). The residues were hydrolyzed with 6 N HCl for 19 h at 100 °C to remove hydrolyzable organic matter. Each dry residue was analyzed three times for carbon and nitrogen content using an elemental analyzer. We believe that formation of nonhydrolyzable artifact from condensation reaction by this separation scheme is negligible or minor since the nonhydrolyzable fraction accounted for only 1% of zooplankton and 8% of phytoplankton when separated by similar chemical treatments (Wang et al., 1998).

2.2. Cross polarization magic angle spinning (CP/MAS) $^{13}$C NMR spectroscopy

A Bruker Avance 400 DPX system with 300W X-nucleus and 100W proton transmitters was used for solid state $^{13}$C NMR spectroscopy. Samples were packed into 7 mm ZrO$_2$ rotors with Kel-F turbine caps and spun at 7 kHz at the magic angle. CP/MAS data were acquired with XWin-NMR 3.0 software package “ceptppm” pulse sequence with a 5 s relaxation time and 1 mS contact time, and proton decoupling during the 53 ms FID (free induction decay) acquisition. From 13,000 to 34,000 scans were accumulated over 16–48 h. Forty hertz line broadening was used for smoothing. Rotor-blank was not subtracted because the blank is a broad hump over the range of 0–200 ppm according to our previous experience. Integration was approximated by cutting and weighing the regions of interest. For inter-comparison purposes, a $^{13}$C NMR spectrum of Washington Lake sediment was also obtained and compared with previously published data (Gélinas et al., 2001a).

2.3. Curie-point pyrolysis gas chromatography/mass spectrometry

Curie-point pyrolysis was performed at 610 °C for 5 s using a Horizon curie-point pyrolyzer with the inlet He pressure at 20 psi and head temperature at 200 °C. The pyrolysis unit was interfaced to an HP 5890-IIA gas chromatograph equipped with RTX-1 (60 m x 3.2 mm x 0.5 μm) column. The oven temperature was programmed to increase from 0 °C (after 5 min hold) to 325 °C at a rate of 3 °C per minute. The flow rate was constant at 5 ml/min. The GC was interfaced to a VG Autospec-Q magnetic sector mass spectrometer operating with electron ionization (50 eV) and 6 kV acceleration of the positive ions in scan mode (m/z 50–650 Da) at nominal mass resolution. Data
were acquired on the Opus software package. Major peaks were identified based on mass spectral comparison with the VG library and NIST library (using Xcalibur software), and comparison of relative retention times with literature values (van de Meent et al., 1980a,b; Hartgers et al., 1992; Ishiwatari et al., 1995; Nguyen et al., 2003).

3. Results and discussion

3.1. Biochemical composition of the nonhydrolyzable fraction of sinking POC

The nonhydrolyzable fractions account for 37% and 44% of sinking POC and SOC samples, respectively (Table 1). Considering potential losses during sample processing, we suspect the actual percentage of the nonhydrolyzable fraction is larger than these values. By demineralization, the organic carbon content of the nonhydrolyzable fraction was concentrated to be 60% and 52% for sinking POC and SOC samples, respectively. The organic carbon to total nitrogen molar ratio (C/N) of this fraction in sinking particulate matter was 33 ± 10 (each sample was analyzed three times), indicating that it is nitrogen poor compared to bulk POC (10 ± 1, Table 1) (Hwang et al., 2004). The large uncertainty of C/N ratio was because of the extremely low N concentration (about 2%) compared to the high C concentration (60%) of the nonhydrolyzable fraction. The δ¹³C value of this fraction of sinking POC was −23.5‰, which was about 2‰ lower than that of the bulk POC (−21.7‰).

¹³C NMR spectra of untreated sinking particulate matter and of the corresponding nonhydrolyzable fraction differ dramatically (Fig. 1 and Table 2). While signals characteristic of common proteins and carbohydrates (45–60 and 60–110 ppm, respectively) were prominent for the bulk particulate matter sample, they diminished significantly in the spectrum for the corresponding nonhydrolyzable fraction. This result clearly indicates that the nonhydrolyzable fraction has a chemical composition that is different from the other major biochemical constituents of the bulk POC, namely proteins and carbohydrates. Two dominant peaks at 0–45 and 110–160 ppm that are characteristic of alkyl and unsaturated carbon (either aliphatic or aromatic), respectively (Hatcher et al., 1983; Wilson, 1987), account for 35% and 31% of the total signal for the nonhydrolyzable fraction (Fig. 1b and Table 2). An NMR spectrum of a nonhydrolyzable fraction isolated from a surface (0.2–0.5 cm) sediment sample collected at a site on the adjacent continental slope resembled that of the Station M deep water sinking POC sample (Fig. 1C). This result suggests that materials comprising the nonhydrolyzable fraction of sinking POC also accumulate in marine sediment. Moreover, the spectrum reported here for the nonhydrolyzable fraction of sinking POC is remarkably similar to those of nonhydrolyzable fractions of sedimentary organic matter from various sources and geologic age, including nonhydrolyzable organic matter in organic-

| Properties of bulk and nonhydrolyzable fraction of sinking POC at Station M, and sedimentary organic carbon (SOC) on the adjacent continental slope (1866 m water depth, 0.2–0.5 cm horizon) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Percentage of   | Org. carbon     | Org. C/total N molar ratio | δ¹³C (‰) | Δ¹⁴C (‰) |
| Bulk sinking POC               | 5.1 ± 0.2       | 10 ± 1          | −21.7 ± 0.2         | −16 ± 10   |
| Nonhydrolyzable fraction of sinking POC | 37              | 60 ± 3          | 33 ± 10             | −23.5 ± 0.1 | −34 ± 20 |
| Bulk SOC                       | 2.5 ± 0.2       | 8 ± 1           | −21.3 ± 0.2         | −157 ± 10  |
| Nonhydrolyzable fraction of SOC | 44              | 52.4 ± 0.3      | 43 ± 1              | −22.2 ± 0.2 | −197 ± 20 |

The uncertainties for C/N molar ratios are standard deviations of triplicate measurements of each sample. Isotope ratios were measured once per each sample and empirical uncertainties for the type of samples were assigned.
Table 2
Distributions of $^{13}$C NMR signal intensity [results for sinking POC from the equatorial pacific are originally from Hedges et al. (2001) and taken from Baldock et al. (2004)]. Assignment of C-structures and functional groups to particular spectral bands is based on Hedges et al. (2002)

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<th>0–45 (%)</th>
<th>45–60 (%)</th>
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<tr>
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<td>8</td>
<td>23</td>
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<td>21</td>
<td>8</td>
<td>10</td>
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<tr>
<td>Nonhydrolyzable fraction of sinking POC</td>
<td>35</td>
<td>7</td>
<td>12</td>
<td>7</td>
<td>23</td>
<td>8</td>
<td>8</td>
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<tr>
<td>Equatorial Pacific sinking POC 0–100 m</td>
<td>44</td>
<td>14</td>
<td>19</td>
<td>2</td>
<td>6</td>
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<td>14</td>
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<tr>
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<td>38</td>
<td>12</td>
<td>21</td>
<td>3</td>
<td>9</td>
<td>1</td>
<td>15</td>
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<tr>
<td>Equatorial Pacific sinking POC 3459 m</td>
<td>40</td>
<td>16</td>
<td>26</td>
<td>3</td>
<td>4</td>
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rich surface sediment from the Mexican coast (Gélinas et al., 2001b), kerogen-like organic matter in sediments from the Black Sea (Garcette-Lepeçq et al., 2000), fossilized algal residue in a Permian Torbanite from South Africa (Knicker et al., 1996), and kerogen from the Carboniferous Puertollano deposit (del Rio et al., 1995).

To further characterize the sinking POC sample we analyzed untreated particulate matter and the corresponding nonhydrolyzable fraction using pyrolysis-GC/MS. Discriminate peaks in the pyrolyzate of the nonhydrolyzable fraction corresponded to long-chain normal hydrocarbons (up to C$_{27}$), benzene and alkylated (C$_1$–C$_3$) benzenes, phenol and alkylated (C$_1$–C$_4$) phenols, pyrrole and alkylated (C$_1$–C$_5$) pyrroles, furan derivatives, pristenes, fatty acids, and phytanes. It should be emphasized that the majority of the GC-amenable pyrolyzate is accounted for by an unresolved complex mixture (UCM), especially for the nonhydrolyzable fraction. There are several key differences between the pyrolyzates of the two samples. In particular, $n$-alkanes and $n$-alkenes (closed and open circles in Fig. 2) are more prominent in the nonhydrolyzable fraction compared to that of the untreated particulate matter. In contrast, the relative amount of carbohydrate-derived compounds (furan, fururaldehydes, and furan derivatives, closed squares in Fig. 2) was much smaller in the nonhydrolyzable fraction compared to untreated particulate matter. Alkylphenols and alkylbenzenes are also more prominent for the nonhydrolyzable fraction than for the untreated particulate matter. The increased relative proportion of aliphatic and aromatic hydrocarbons in the pyrolyzates of the nonhydrolyzable fraction is consistent with the strong signal for alkyl and unsaturated/aromatic carbon in the $^{13}$C NMR spectrum.

3.2. Potential sources and preservation of the nonhydrolyzable fraction

Hydrogen-rich lipids have lower $\delta^{13}$C values than carbohydrates and amino acids because of isotopic fractionation during their biosynthesis (Degens et al., 1968; DeNiro and Epstein, 1977; Hayes, 2001). The $\delta^{13}$C values of the nonhydrolyzable fraction of both sinking POC and SOC are lower by 1.8/\text{per mil} and 0.9/\text{per mil} respectively, than those of the corresponding bulk organic matter (Table 1). The strong $^{13}$C NMR signal for alkyl and unsaturated/aromatic carbon of the nonhydrolyzable fraction and dominance of aliphatic and aromatic compounds in the pyrolyzates are consistent with depleted $\delta^{13}$C values of lipids that are lower than those of total hydrolyzable amino acids and neutral carbohydrates isolated from the same samples (Hwang and Druffel, 2003). Together, these observations suggest that a major component of the nonhydrolyzable fraction may share a common biochemical origin with lipids.

Highly aliphatic, chemically inert residues have previously been isolated from certain plants, including micro-algae (Tegelaar et al., 1989a). The aliphatic nature of these “resistant biopolymers,” termed algaenan, has been shown from both spectroscopic ($^{13}$C NMR, FT-IR) and degradative (pyrolysis, RuO$_4$ oxidation cleaving ether bonds) studies (Largeau and de Leeuw, 1995; Blokker et al., 1998; Tegelaar et al., 1989b). In addition to aliphatic biopolymers, alklyphenols were observed in the pyrolyzates of the resistant residues of various macroalgae (van Heemst et al., 1996). Accumulation of these alklyphenols with increasing depth was also observed in high molecular weight dissolved organic matter in the North Pacific Ocean (van Heemst et al., 1993) and particulate organic matter in the Mediterranean Sea (Peulvè et al., 1996). Thus, it seems plausible that the resistant residues of these organisms may contribute to (or be the precursors for) the nonhydrolyzable fraction of sinking POC in the deep ocean. However, since known species of algaenan-producing phytoplankton are not currently recognized as major primary producers in open ocean settings, the specific biological precursors for the aliphatic compounds, as well as alklyphenols, and alkylbenzenes remain unknown.

The existence of compounds such as alkylpyrroles in the pyrolyzate of the nonhydrolyzable fraction suggests that resistant aliphatic biopolymers may not be the only component. These nitrogen-containing compounds might originate from protein residues (Ishiwatari et al., 1995) that evaded chemical attack by protection within refractory macromolecular networks (Nguyen et al., 2003), melanoidin-type precursors (Peulvè et al., 1996), or covalently bound tetrapyrrole pigments (Sinninghe Damste et al., 1992). Pristenes might originate from tocopherols (Goossens et al., 1984) or from chlorophylls.
Black carbon, produced during biomass burning and transported by winds and rivers to the surface ocean, represents another potential source of nonhydrolyzable organic matter. Black carbon from fossil fuel burning (devoid of $^{14}$C) can be excluded as a major source (<10%) because the observed radiocarbon content of the nonhydrolyzable fraction of POC is close to modern values (Wang et al., 1998; Hwang and Druffel, 2003). Residues from biomass burning could, however, partly account for aromatic moieties detected in the nonhydrolyzable fraction (Figs. 1b, c and 2b). Improved quantification and characterization of black carbon in sinking POC is required to better constrain its contribution.

The nonhydrolyzable fraction accounts for about 40% of sinking POC at 3450m depth. However, it accounts for only a minor fraction of plankton [1% of zooplankton and 8% of phytoplankton (Wang et al., 1998)]. Therefore, the nonhydrolyzable fraction either accumulates during vertical transit to the deep ocean or has additional sources such as laterally advected resuspended SOC. Artifact formation during the chemical processing can be eliminated as an explanation because it would produce an equivalent amount of artifact for plankton samples, yet the nonhydrolyzable fraction accounts for significantly smaller fractions of those samples.

Entrainment of resuspended sediment from the adjacent margin into sinking POC intercepted by deep sediment traps would potentially increase the abundance of the nonhydrolyzable fraction at Station M. The California margin receives kerogen eroded from marine-origin sedimentary rock via small mountainous rivers (Masiello and Druffel, 2001; Blair et al., 2003; Komada et al., 2004). Kerogen can exhibits similar $\delta^{13}$C values to those of lipids (Komada et al., 2005). However, $\Delta^{14}$C values of the nonhydrolyzable fraction exclude kerogen as a major contributor to sinking POC. Isotopic mass balance based on radiocarbon values of the nonhydrolyzable fraction (Table 1) suggests that up to 36% of this fraction would have had to originate from resuspended sediment if it were the only source of $^{14}$C-depleted organic carbon. This would imply that 24% of sinking POC comprises the nonhydrolyzable material derived from organisms in the overlying water column.

DOC represents another potential source of $^{14}$C-depleted carbon to sinking POC (Druffel and Williams, 1990; Hwang et al., 2001).

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Fig. 2. Total ion chromatogram of pyrolyzate of (a) bulk sinking POC and (b) the corresponding nonhydrolyzable fraction. Numbers over alkanes/alk-1-enes are number of carbons in the alkyl chain.
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et al., 2006). However, because DOC is more $^{14}$C-depleted than SOC ($\Delta^{14}C = -550$‰ in deep waters at Station M (Bauer et al., 1998)), the incorporation of DOC would require that a higher percentage of the nonhydrolyzable POC originated from plankton in the overlying water column.

Hence, selective accumulation of a nonhydrolyzable fraction derived from surface ocean productivity appears the most plausible explanation for its high relative abundance in the deep ocean. This appears inconsistent with the hypothesis of nonselective preservation (Hedges et al., 2001). One explanation for the contrasting conclusions of this study and the Hedges et al.’s study is that the extent of selective preservation may differ from one oceanic environment to another. Supporting this interpretation, our $^{13}$C NMR spectrum of sinking POC appears significantly different from those in the Equatorial Pacific and the Arabian Sea at all sampling depths. Specifically, the signal at 110–160 ppm (31%) is stronger for sinking POC at Station M than corresponding samples from the Equatorial Pacific (4–10%, Table 2). We do not believe that this difference was caused by instrumental conditions affecting data acquisition because the spectrum of a Washington Lake sediment sample obtained for inter-comparison purposes was virtually identical to the published result (Gélinas et al., 2001a).

The composition of sinking POC in the deep ocean may be similar to that of the underlying SOC considering that chemical composition determined by molecular analysis does not change significantly between sinking POC in the deep ocean and SOC (Wakeham et al., 1997). The abundances of the nonhydrolyzable fraction in sinking POC at Station M and SOC on the California margin were similar, also suggesting that compositional change at the water–sediment boundary is small. The proportion of nonhydrolyzable organic matter in California margin surface sediments (44%) was comparable to that in Mexican and Peruvian coastal surface sediments, about 50%, as estimated by the sum of the $^{13}$C NMR signal of nonprotein alkyl and black carbon (Gélinas et al., 2001b). However, the proportion of nonhydrolyzable organic matter estimated for Equatorial Pacific surface sediments, and for Arabian Sea sediments was significantly lower [29% and 36%, respectively (Gélinas et al., 2001b)]. This suggests that there may be geographic variation in the accumulation of nonhydrolyzable organic matter, presumably tied to differences in organic matter supply and depositional environment. Oxygen exposure time was suggested as a major controlling factor for selective preservation of the nonhydrolyzable fraction (Gélinas et al., 2001b). However, similarities in the relative abundance of the nonhydrolyzable fraction in sinking POC and sediments (Baldock et al., 2004) implies that the composition of source organic matter, or sinking POC may be a crucial controlling factor of the composition of SOC.

4. Conclusions

$^{13}$C NMR spectroscopy and analytical pyrolysis indicate that the composition of nonhydrolyzable organic matter, which accounts for about 40% of deep sinking POC in the northeast Pacific, is distinct from that of bulk POC. Curie-point pyrolysis-GC/MS analysis of the nonhydrolyzable fraction showed that this fraction is enriched in aliphatic and aromatic moieties and depleted in carbohydrate-derived compounds compared to bulk POC. The prominence of aliphatic and aromatic compounds in the pyrolyzate of the nonhydrolyzable fraction is consistent with the strong signal for alkyl and unsaturated/aromatic carbon in the corresponding $^{13}$C NMR spectrum. Results from both analyses reinforce prior interpretations that the nonhydrolyzable fraction is largely composed of “lipid-like” material based on its carbon isotope ratios (Hwang and Druffel, 2003). Although the biological source(s) of the nonhydrolyzable fraction remains elusive, selective accumulation of resistant components of organisms is a feasible mechanism for its formation.

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References


