

Why Cretaceous black shales have high C/N ratios: Implications from SEM-EDX observations for Livello Bonarelli black shales at the Cenomanian-Turonian boundary

Naohiko Ohkouchi¹, Jun'ichiro Kuroda², Makoto Okada³ and Hidekazu Tokuyama²

¹ Research Program for Paleoenvironment, Institute for Frontier Research on Earth Evolution (IFREE)

² Ocean Research Institute, University of Tokyo, 1-15-1 Mianamidai Nakano-ku Tokyo 164-8639, Japan

³ Department of Environmental Sciences, Ibaraki University, 2-1-1 Bunkyo, Mito 310-8512, Japan

Introduction

A conspicuous chemical characteristic of Cretaceous black shales is that they substantially concentrate organic carbon relative to nitrogen compared with organic matter produced in the modern surface ocean (around 8; Watson and Whitfield, 1985). Carbon to nitrogen weight ratios (C/N ratios) of sedimentary organic matter have been used as a semi-quantitative indicator to infer the contribution of terrestrial organic matter to the sediments, since lignin and cellulose, structural components in terrestrial vascular plants contain little nitrogen, and thus organic matter produced on land has substantially elevated C/N ratios on average (generally over 20). In many black shales investigated so far, however, direct and indirect evidence like terrestrial biomarkers and depositional settings have suggested minor terrestrial input (e.g., Meyers et al., 1984). Furthermore, the C/N ratios of Cretaceous black shales deposited in pelagic environments also show extraordinarily high C/N ratios (Table 1). Therefore, elevated C/N ratios could characterize most organic matter in Cretaceous black shales.

Based on evidence from sedimentary organic molecules and nitrogen isotopic compositions, Ohkouchi et al. (1997) proposed that the major primary producer during Oceanic Anoxic Event was diazotrophic cyanobacteria. In the present study, we investigated black shale using scanning electron microscopy-energy dispersion X-ray spectrometry (SEM-EDX) to investigate the distribution of elements, especially carbon and nitrogen. The SEM-EDX technique allows us to determine mean elemental compositions for an area as small as 1 μm in diameter. As far as we know, this is the first application of this technique to investigate the origin of organic matter in black shales. Here we focus our discussion on C/N ratios of organic matter in black shales deposited in northern Tethys at the Cenomanian-Turonian Boundary, Cretaceous period.

Samples and methods

Our samples have been described in Ohkouchi et al. (1997; 1999) and Kuroda (2002). Briefly, rock samples from Livello Bonarelli black shales (Cenomanian-Turonian boundary, Cretaceous, 93.5Ma; Gradstein et al., 1994) were collected at Gorgo Cerbara in the northern Apennines, Italy. To avoid potential contamination, we sampled as deeply as possible from the exposure. They were pulverized, and then contents of total organic carbon and total nitrogen were determined by a Fisons NA1500 NCS analyzer after the samples were acidified

with sufficient amount of 6M HCl, and washed with distilled water several times.

For SEM-EDX observation we selected sample GCB19-2-7, because its TOC content is higher than 20%. Observations by a field emission SEM-EDX were performed with a Hitachi S-4700 equipped with Horiba EX-200 energy dispersion X-ray spectrometry at Hitachi-Science-Systems, Ltd. Measurements were done under an accelerating voltage of 5-15kV. The surface of the sample was not coated. Experimental details will be given elsewhere (Ohkouchi et al., in prep.).

Results and discussion

In Livello Bonarelli black shales, total organic carbon content of bulk samples range from 2.6 to 26.3%, and the C/N ratios are 31.5 ± 2.4 (1σ , $n=8$). Under the SEM, organic matter is observed as dark-colored material. Based on morphology, it can be classified into three categories (Fig. 1); a) flat-shaped material with a diameter of 5-20 μm and a thickness of less than 1 μm , b) fragmental material with many spores, and c) sack-shaped material with a diameter of around 10 μm and commonly containing pyrite crystals. In terms of C/N ratios, these amorphous organic matter in the black shales could be classified into two endmembers. Organic matter categories a and b have C/N ratios of 2-7 (organic matter A), whereas that of category c is infinite (no nitrogen is contained; organic matter B). In such a two-endmember system, the C/N ratios of total organic matter ($(C/N)_{\text{TOM}}$) can be mathematically formulated as;

$$(C/N)_{\text{TOM}} = (C/N)_A (1 + (1-f) / f [C]_B / [C]_A),$$

where f is a fraction of the A in total organic matter ($0 \leq f \leq 1$), and $[C]_A$ and $[C]_B$ are concentrations of carbon in organic matter A and B, respectively. $(C/N)_A$ represents the C/N ratio of A, and here we set it to be 5 based on the observations described above. Fig. 2 illustrates the relationship between $(C/N)_{\text{TOM}}$ and $[C]_B / [C]_A$ ratios. Nitrogen content in amorphous organic matter in geological samples is generally only a few percent or less (Tissot and Welte, 1984). Therefore, if we assume the elemental composition of organic matter B to be the same as A except for nitrogen, the carbon concentration in B should be slightly higher than that of A. Therefore, we set the $[C]_B / [C]_A$ ratio of Livello Bonarelli black shale to be close to or somewhat larger than unity. The data in Fig. 2 suggest the a fraction of N-rich component in the black shale would be 0.15 to 0.2; in other words, 80 to 85% of organic matter in the

Bonarelli black shales would be composed of the N-depleted sack-shaped organic matter.

What is the source of the N-depleted, sack-shaped organic matter? Nucleic acids and proteins are major N-rich components of organisms. Although microbial and chemical processes in the water column and sediments substantially modify the chemical structure of organic matter, the C/N ratio is a relatively conservative number, and as far as fresh (no high P-T alteration) sediments are concerned, in most cases it mainly reflects the original value. Therefore, the N-depleted organic matter would have originally been N-depleted carbohydrates or lipids. Although we do not have convincing evidence, we speculate it to be the cell walls of heterocysts in cyanobacteria. Our speculation is based on the morphology and chemical composition of cell walls for modern cyanobacterial heterocysts. The heterocyst is a specialized cell for fixing gaseous dinitrogen and it is generally 10-20 μm in diameter, with a cell wall thickness of as thick as a few μm . The cell wall of the heterocyst is made of a laminated glycolipid layer with a thin polysaccharide layer on its outer surface (Adams, 1997) to prevent oxygen diffusing into the cell which would significantly reduce nitrogenase activity (e.g., Berman-Frank et al., 2001). These glycolipids and polysaccharides lack nitrogen (i.e., $\text{C/N}=\infty$) and are relatively resistant to microbial attack, and thus they are potentially preserved in the sediments.

At present we have no evidence to suggest that what we have observed and discussed here can be extrapolated to "black shales" deposited in other time periods. Nijenhuis et al. (1996) showed that C/N ratios of Miocene Mediterranean sapropels ranged from 15 to 18 which is substantially higher than those of adjacent rocks. Ingall et al. (1993) also showed that C/N ratios of black shales from late Devonian to early Mississippian sediments were mostly confined to 20-25. In Precambrian black shales, the C/N ratio is commonly higher than 20 (Yamaguchi, 2002). To investigate the heterogenic distribution of nitrogen in black shales, further, SEM-EDX and EPMA observations are now underway.

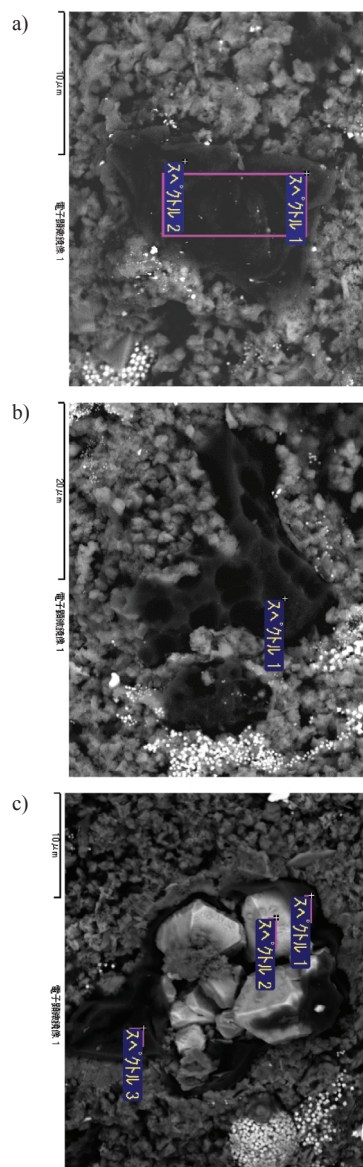
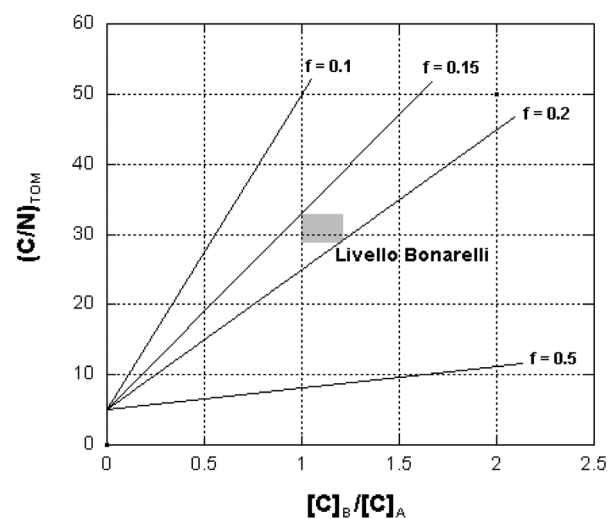
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Table 1. Compilation of total organic carbon to total nitrogen weight ratio (C/N ratio) of Cretaceous black shales and modern sediments.

Sample	Material	Location	Age (Ma)	C/N ratio	n	Reference
<i>Cretaceous black shale</i>						
Livello Bonarelli	Bulk	N Thethys	93 Ma	31	8	This study
DSDP 530	Bulk	SE Atlantic	93 Ma	36 - 40	3	Meyers <i>et al.</i> (1984)
Thalmann	Bulk	Central Pacific	116 Ma	18 - 43	7	N. Ohkouchi and W. V. Sliter, unpub. data
DSDP site 367,368	Kerogen	NE Atlantic	Early-Late Cret.	22 - 31	6	Deroo <i>et al.</i> (1978)
DSDP site 603	Bulk		Upper Cret.	32 - 72	5	Joyce and van Vleet (1986)
<i>Recent sediments</i>						
175°E transect	Bulk	Central Pacific	0-10 kyr	4.5 - 11	22	Ohkouchi <i>et al.</i> (1997)
KH92-1-5cBX	Bulk	Western Eq. Pacific	0-20 kyr	5.5 - 16	15	Ohkouchi (1995)
OCE326-GGC5	Bulk	Bermuda Rise	0-23 kyr	3.0 - 10	186	N. Ohkouchi and T. Eglinton, unpub. data

**Figure 1.** Scanning electron microscope images of three kinds of organic matter in the Livello Bonarelli black shales (GCB19-2-7; Cenomanian-Turonian Boundary, Cretaceous period) collected from the northern Apennines, Italy. Dark regions in these images are organics. a) Flat-shaped, b) fragment with many spores, and c) sack-shaped organic matter.**Figure 2.** Carbon to nitrogen ratios in total organic matter ((C/N)_{TOM}) as a function of [C]_B/[C]_A ratio. The term *f* is a fraction of organic matter A (C/N ratio=5). Shaded area indicates the region where Livello Bonarelli black shale samples would plot.

