Chapter

17

Isotopomers of chlorophyll nuclei: Theories and an application

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Abstract

In this review, we discuss site-specific carbon isotopic compositions of chlorophylls with special reference to their biosynthetic pathways in the cell. The carbon isotopic composition is ultimately inherited from glutamic acid, a major amino acid in the cell. Intramolecular (site-specific) carbon isotopic distributions of geoporphyrins, geological derivatives of the tetrapyrrole nuclei of various chlorophylls in sediments and sedimentary rocks, are thus potentially useful for investigating the carbon cycle in the geological past. As a case study, we report analytical results for the mean carbon isotopic compositions of the methine bridge carbons (i.e., C−5, C−10, C−15, and C−20 positions in the tetrapyrrole structure) in geoporphyrins derived from chlorophylls *a* and *c* in the Miocene Onnagawa Formation, Japan. The results suggest that β-carboxylation was not an important process for carbon assimilation of photoautotrophs during the formation of this sediment. Although substantial biogeochemical information is recorded as isotopomeric signatures of sedimentary tetrapyrroles, we are still just beginning to explore its potential, and further experiments are necessary to establish this approach as a robust tool for biogeochemistry.

INTRODUCTION

This review concerns with the isotopomers (i.e., site-specific or intramolecular carbon isotopic compositions) as well as bulk carbon isotopic compositions of chlorophyll and

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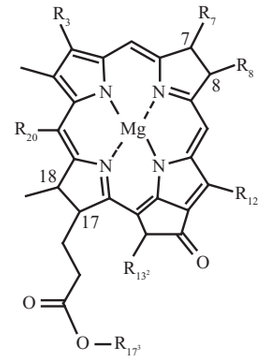
bacteriochlorophyll nuclei in terms of metabolic processes. Among the various types of tetrapyrroles produced by organisms, chlorophylls are far more abundant, widespread, and obvious biological molecules in photoautotrophic organisms. Chlorophyll is an essential part of the photosynthetic apparatus and functions as a light energy-harvesting pigment.

The advantages of chlorophyll as a tool for biogeochemical and geological studies are threefold. First, chlorophylls have various chemical structures that are correlated with major photoautotrophic groups. Therefore, the structural variations and relative abundances of chloropigments in the natural environment provide first-order diagnostic information on the components of the photoautotrophic community. For example, if abundant chlorophyll *b* or its derivatives are observed in a given horizon of lake sediment, you can tell that the green algae were among the primary photoautotrophs in the lake when the sediment was formed. Second, chlorophyll contains both carbon and nitrogen atoms whose stable isotopic compositions (i.e., $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios) provide valuable information about biogeochemical processes associated with assimilation and metabolism of these elements, allowing reconstruction of the surface water environment where the compounds were produced. Because most biomarkers widely used for investigation of the contemporary environment or paleoenvironment do not contain nitrogen, nitrogen isotopic record of chlorophylls uniquely contributes to understanding environmental processes related to the nitrogen cycle. Third, the tetrapyrrole nuclei of chlorophylls are well preserved in sediments and sedimentary rocks, oil shales, and even crude oil (e.g., Treibs 1934, 1936; Blumer 1965; Baker and Louda 1986; Eckardt et al. 1991; Callot and Ocampo 2000; Kashiyama et al. 2007b). Therefore, they are of particular interest for reconstruction of biogeochemical processes in the geological past. Despite these merits, chlorophyll abundance and their isotopes have been measured in relatively a limited number of studies from only a few laboratories (Hayes et al. 1987; Madigan et al. 1989; Popp et al. 1989; Katase and Wada 1990; Bidigare et al. 1991; Chicarelli et al. 1993; Sachs et al. 1999; Beaumont et al. 2000; Ohkouchi et al. 2006; Sato et al. 2006). It is mainly because 1) it requires time-consuming, tedious chemical/analytical procedures for isolation and purification of the chlorophylls (and their derivatives) from the complex mixtures of organic molecules in natural materials, and 2) the measurement of stable isotopic compositions can only be achieved by a classical off-line method rather than a conventional on-line method (e.g., Hayes et al. 1990; Meier-Augenstein 1999).

We have recently improved the isolation/purification protocol for chlorophylls and their diagenetic derivatives from sediments and particulate organic matter in the water column for precise, rapid determination of carbon and nitrogen isotopic compositions (e.g., Ohkouchi et al. 2005; Chikaraishi et al. 2005, 2008; Kashiyama et al. 2007a). Although the method still employs (automatic) fraction collection of chlorophylls using high-performance liquid chromatography (HPLC) followed by off-line measurement of isotopic compositions, it provides a strict analytical basis for studying the utility of these signatures. We have applied this method to several sedimentary rocks to better understand the surface water environment during their formation in the geological past (Kashiyama et al. 2008a, b). These results



	R_3	R_7	R_8	7, 8-bond	R_{12}	$R_{13}{}^2$	R_{20}	$R_{17}{}^3$	17 ,18-bond
Chl *a*	$-CH=CH_2$	Me	Et	double	Me	$-COO-CH_3$	H	phytyl	single
Chl *b*	$-CH=CH_2$	-CHO	Et	double	Me	$-COO-CH_3$	H	phytyl	single
Chl *c*	$-CH=CH_2$	Me	Et	double	Me	$-COO-CH_3$	H	H	double
Chl *d*	-CHO	Me	Et	double	Me	$-COO-CH_3$	H	phytyl	single
BChl *a*	$-CO-CH_3$	Me	Et	single	Me	$-COO-CH_3$	H	phytyl	single
BChl *b*	$-CO-CH_3$	Me	$=CH-CH_3$	single	Me	$-COO-CH_3$	H	phytyl	single
BChl *c*	$-CHOH-CH_3$	Me	Et, Pr, iB	double	Me, Et	H	Me	famesyl	single
BChl *d*	$-CHOH-CH_3$	Me	Et, Pr, iB	double	Me, Et	H	H	famesyl	single
BChl *e*	$-CHOH-CH_3$	-CHO	Et, Pr, iB	double	Et	H	Me	famesyl	single
BChl *g*	$-CH=CH_2$	Me	$=CH-CH_3$	single	Me	$-COO-CH_3$	H	famesyl	single

Fig. 1. Chemical structures of representative chlorophylls (*a*, *b*, c_1, and *d*) and bacteriochlorophylls (*a*, *b*, c_{1-3}, d_{1-3}, e_{1-3}, and *g*). All chlorophylls are composed of four pyrroles, or "tetrapyrrole."

strongly suggested that diazotrophic (i.e., N_2-fixing) cyanobacteria were significant primary producers during the formation of certain petroleum source rocks.

In this review, we focus on the topic how the carbon isotopic composition of chlorophylls is controlled in the photoautotrophic cell, a fundamental concern when interpreting the isotope record of tetrapyrrole structures in natural materials including sediments. Furthermore, we demonstrate that the intramolecular carbon isotopic compositions of chlorophylls and their derivatives, such as sedimentary porphyrins (sometimes called geoporphyrins, petroporphyrins, or metalloalkylporphyrins), can provide critical information and profound insights into biogeochemical processes.

Chlorophylls and bacteriochlorophylls: structures and distribution

We begin with a brief overview of the chemical structures and natural distributions of

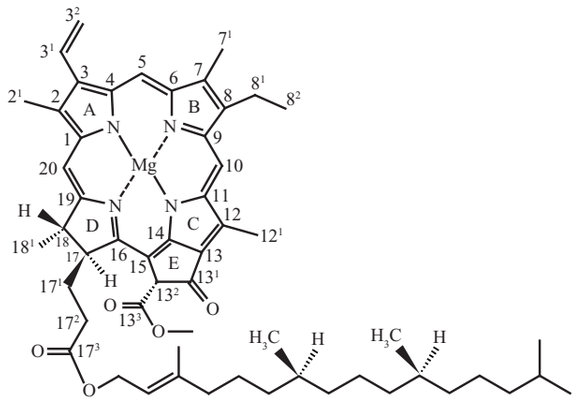


Fig. 2. The numbering scheme for chlorophyll *a* proposed by the International Union of Pure and Applied Chemistry (IUPAC). The same basic numbering scheme applies to all chlorophylls and bacteriochlorophylls.

chlorophylls, an important group of tetrapyrroles (i.e., compounds containing four pyrroles or nitrogen-containing five-membered rings) produced in the cell. The chlorophylls vary widely in chemical structure, from chlorophyll *a* to *d*, and from bacteriochlorophyll *a* to *g* (Fig. 1). With further variations within each of these chloropigments, nearly 100 chlorophylls are known today. In Fig. 2, we illustrate the atom numbering schemes for chlorophylls based on that introduced by International Union of Pure and Applied Chemistry (IUPAC) in 1979. The five rings of chlorophylls and bacteriochlorophylls are lettered A − E, and the carbon atoms of the macrocycle are numbered clockwise, beginning with ring A. In most cases, a magnesium ion is at the center of the molecule.

Among the various chlorophylls, chlorophyll *a* is found in all known eukaryotic, oxygenic photoautotrophs (Table 1), serving as the primary light-harvesting antenna pigment as well as a reaction center for capturing solar energy. Currently, the only known exception to this rule is the cyanobacterium *Acaryochloris marina*, which produces chlorophyll *d* as its sole chloropigment (Miyashita et al. 1996). In most marine microalgae, 0.25−5% of cellular carbon is associated with chlorophyll *a* (Geider 1987).

Chlorophyll *b* is the second-most important chlorophyll in terrestrial environments. Chlorophyll *b* differs from chlorophyll *a* only by one of the functional groups at the C−8 position (a -CHO group in place of a CH_3-group, Fig. 1). In both chlorophylls *a* and *b*, a 20-carbon diterpenoid alcohol group (phytol) is esterified to the C−17 propionic acid attached to ring D. Higher plants and green algae contain chlorophyll *a* and chlorophyll *b* at a ratio of ~3: 1 (Table 1). Chlorophyll *b* acts as an accessory pigment that participates directly in the light reactions of photosynthesis.

Chlorophyll *c* homologues (i.e., chlorophylls c_1, c_2, and c_3) structurally differ from

Table 1 Distribution of chlorophylls and bacteriochlorophylls in photoautotrophs (modified from Blankenship (2004))

Group	Chlorophyll				Bacteriochlorophyll					
	a	*b*	*c*	*d*	*a*	*b*	*c*	*d*	*e*	*g*
Cyanobacteria	+			−						
Green algae	+	+								
Diatoms	+		+							
Brown algae	+		+							
Dinoflagellate	+		+							
Cryptomonads	+		+							
Red algae	+									
Higher plants	+	+								
Purple bacteria					+	+				
Green sulfur bacteria					−		+	+	+	
Green non-sulfur bacteria					−		+	+	+	
Heliobacteria										+

-Limited distribution

other chlorophylls in that they have a fully unsaturated, symmetric porphyrin nucleus rather than a chlorin nucleus (e.g., Zapata et al. 2006). These chlorophylls have propenoic acid at the C−17 position, instead of the phytol esterified propionic acid side chain of the other chlorophylls (Fig. 1). Chlorophyll *c* occurs in the primary aquatic photoautotrophs, including diatoms, dinoflagellates, brown algae, and haptophytes, accompanying chlorophyll *a* (Table 1).

Chlorophyll *d* was originally discovered in red macroalgae over 60 years ago, as the fourth type of chlorophyll (Manning and Strain 1943). The molecular structure of chlorophyll *d* is similar to chlorophyll *a*; a vinyl group at the C−3 position of chlorophyll *a* is replaced with a formyl group (Fig. 1). However, it was long erroneously considered a laboratory artifact. In 1996, it was re-discovered in the symbiotic cyanobacterium *Acaryochloris marina* from coralline (Miyashita et al. 1996). Recently, Kashiyama et al. (2008c) reported it in various natural aquatic environments, including freshwater and hypersaline lakes, strongly suggesting an ubiquitous distribution in the aquatic environment.

Bacteriochlorophylls are found in prokaryotic photosynthetic bacteria and heliobacteria. These photoautotrophs conduct anoxygenic photosynthesis; they use hydrogen sulfide rather than water as an electron donor, and thus produce elemental sulfur (S^0) rather than molecular oxygen (O_2) as the byproduct of photosynthesis. Purple bacteria synthesize either bacteriochlorophyll *a* or *b* (Table 1). These bacteriochlorophylls are different from chlorophylls in that they have a bacteriochlorin rather than chlorin nucleus (Fig. 1). Furthermore, they contain a C_{20} phytol side chain rather than a C_{15} farnesol.

Bacteriochlorophylls *c*, *d*, and *e* are produced only by green photosynthetic bacteria, all

of which are strictly anaerobic (Table 1). Although they are termed "bacteriochlorophylls", their ring B contains a C−7-C−8 double bond as in chlorophylls; thus, these pigments are actually chlorins rather than bacteriochlorins (Fig. 1). Bacteriochlorophylls *c* and *e* have a methyl substituent at the C−20 methine bridge carbon (Glaeser et al. 2002). Bacteriochlorophyll *g* has a bacteriochlorin nucleus and is produced only by strictly anaerobic heliobacteria (Brockmann and Lipinski 1983).

Biosynthesis of tetrapyrroles in the cell

It is essential to comprehend the biosynthetic processes of chlorophylls in the cell for proper understanding of intramolecular isotopic signatures. The biosynthetic pathways of chlorophylls together with the bifurcation points for the syntheses of other tetrapyrroles including hemes, bilins, and corrins are outlined in Fig. 3. The biosynthetic processes of these tetrapyrrole structures in the cell have been intensively studied for more than 60 years. It has been demonstrated that 5-aminolevulinic acid or δ-aminolevulinic acid (ALA, Fig. 3) is a universal precursor in the biosynthesis of all tetrapyrrole compounds produced in the cell (e.g., Kikuchi et al. 1958; Beale 1993; Porra 1997).

There are two distinct mechanisms of ALA formation (Fig. 3). In mammals, yeasts, and fungi, ALA is formed from glycine and succinyl-CoA in a one-step condensation reaction catalyzed by the enzyme ALA synthase (ALAS). Because of the pioneering studies of David Shemin, who ingested ^{15}N-labeled glycine to determine the half-life of hemoglobin (Shemin and Rittenberg 1946), this pathway is often called the "Shemin pathway". In contrast, in plants, algae, and most bacteria, ALA is synthesized from L-glutamic acid in multi-enzyme-catalyzed reactions (Fig. 3; e.g., Beale et al. 1975; Meller et al. 1975; Beale 1995). Glutamic acid is activated to glutamyl-tRNA in the first step, is then reduced to glutamate−1-semialdehyde (GSA), and subsequently forms ALA by GSA aminotransferase. This unique pathway is traditionally referred to as the "C5 pathway". However, it has been demonstrated that in some photosynthetic organisms including the α subgroup of purple bacteria and *Euglena*, ALA is produced through the Shemin pathway (Beale et al. 1981; Iida et al. 2002). Biosynthesis of ALA is known to be a rate-limiting step in the biosynthesis of tetrapyrroles. The activities of ALAS and GSA aminotransferase are strictly regulated by feedback inhibition.

In the Shemin pathway, the carbon skeleton of ALA is inherited from both succinyl-CoA and glycine; carbon atoms at the C−1 to C−4 positions of ALA derive from the C−1 to C−4 carbons of succinyl-CoA, whereas the C−5 position of ALA is derived from the C−2 position of glycine (Fig. 3). In contrast, the carbon skeleton of ALA produced through the C5 pathway is inherited only from glutamic acid. In this case, the carbons at positions C−1 to C−5 of ALA derive from the C−5 to C−1 positions of glutamic acid. In the final reaction of the C5 pathway, GSA is the only required substrate and ALA is the only product. Therefore, the stable carbon isotopic compositions at each of the positions in ALA should reflect those

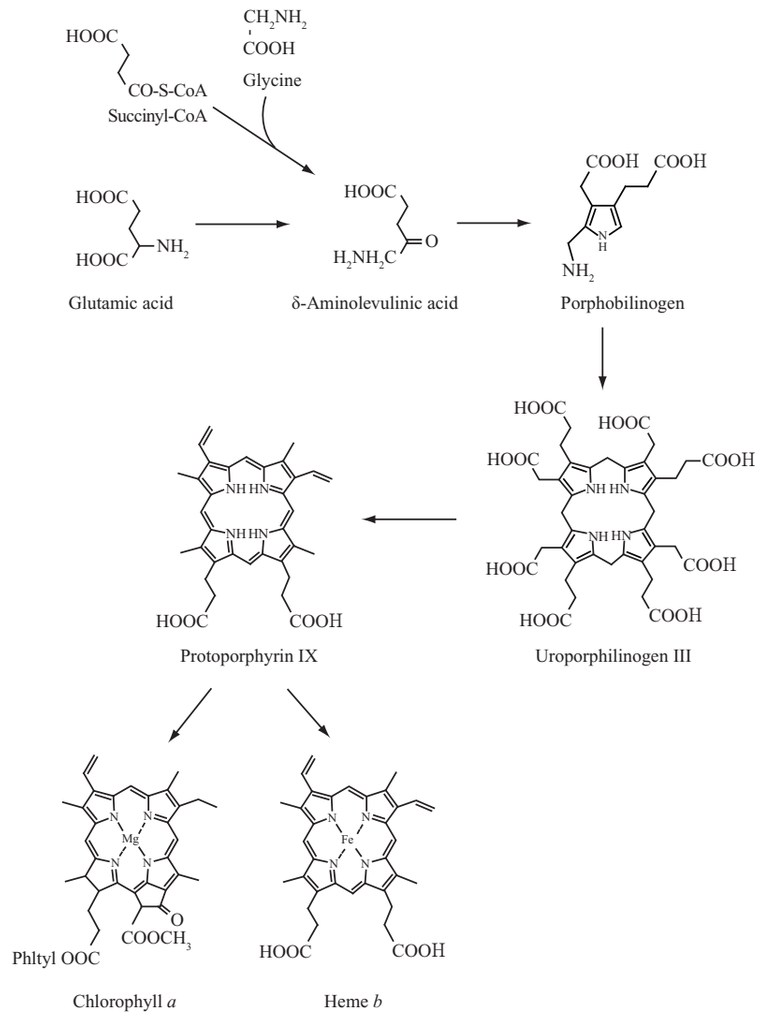


Fig. 3. Overview of the biosynthetic pathways of chlorophylls and hemes.

of glutamic acid.

Once ALA is formed, two molecules of ALA condense to form porphobilinogen in a process catalyzed by ALA dehydratase (Fig. 3). Four molecules of porphobilinogen are further condense to form tetrapyrrole uroporphyrinogen III (Fig. 3). Overall, eight molecules of ALA are condensed to form the tetrapyrrole structure of chlorophylls. Uroporphyrinogen III then undergoes a series of decarboxylation and reduction reactions,

which are followed by insertion of the metal ion (i.e., Mg^{2+}, Fe^{2+}, or Zn^{2+}) into the tetrapyrrole ring to form protoporphyrin IX (Fig. 3). The biosynthetic pathway of Fe-chelated heme branches at this point. In chlorophyll synthesis, metallation is followed by construction of the isocyclic ring E, reduction of ring D, and attachment of the phytyl (or farnesyl) side chain (Fig. 1).

The same basic pathway is followed for synthesis of all types of chlorophylls and bacteriochlorophylls. The biosynthetic processes of the various chlorophylls branch in the final stages of chlorophyll formation. For example, chlorophyll *b* is synthesized by oxidation of the methyl group at the C−7 position to give a formyl group.

In the bacteriochlorophyll *a* synthesis, the vinyl group at the C−3 position is converted to an acetyl group in the final step. However, this process does not cleave the carbon skeleton; thus, the carbon isotopic composition of bacteriochlorophyllide *a* is the same as that of the immediate precursor. In the synthesis of the bacteriochlorophyll *e* homologues, additional carbon atoms are added to the C−8[2] and C−20 positions in the final steps (Senge and Smith 1995). It has been demonstrated that these extra carbon atoms in the bacteriochlorophylls *e* are derived from the methyl group of S-adenosylmethionine, a metabolic product of methionine.

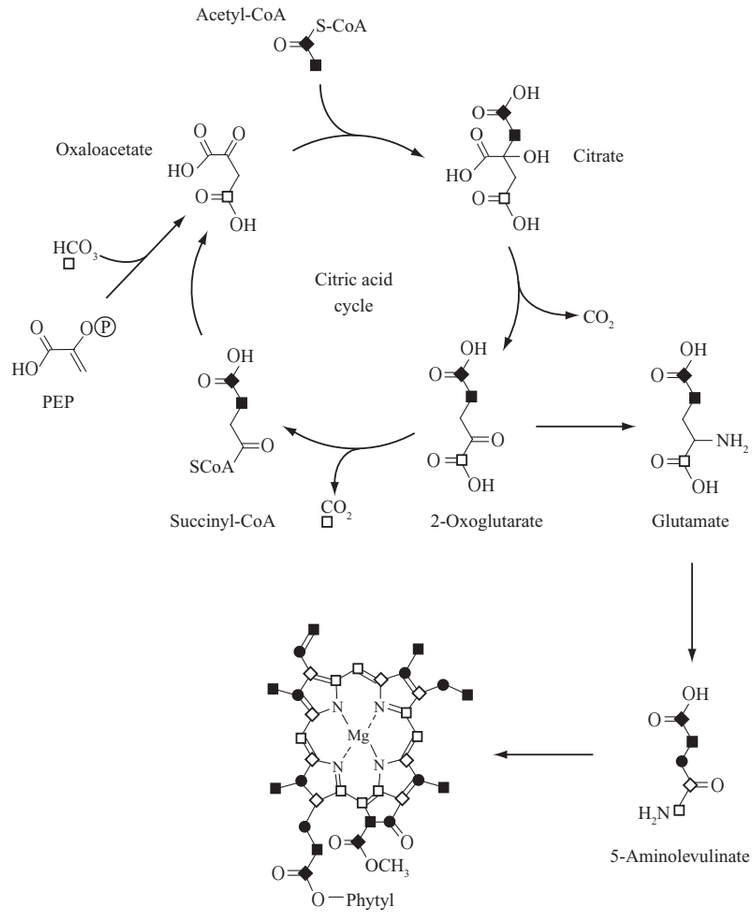
Intramolecular carbon isotopic compositions of chlorophylls

The biosynthetic pathways of chlorophyll synthesis described above indicate that the five carbon atoms in glutamic acid are not equally incorporated into the chlorophyll nucleus. For a chlorophyll *a* nucleus (i.e., chlorophyllide *a*), eight carbon atoms from positions C −1 through C−4 in glutamic acid are introduced, whereas only two carbon atoms from the C−5 position of glutamic acid are incorporated (Fig. 4). We should note that the methoxy group carbon (-OCH_3) at the C−13[2] position originates from a methyl group (me) derived from the S-adenosylmethionine pool. Overall, in a mechanistic sense, the carbon isotopic composition of the chlorophyll *a* nucleus (chlide *a*) can be expressed by the following equation (Ohkouchi et al. 2008):

$$^{13}\delta_{\text{chlide } a} = (8^{13}\delta_{C-1} + 8^{13}\delta_{C-2} + 8^{13}\delta_{C-3} + 8^{13}\delta_{C-4} + 2^{13}\delta_{C-5} + {}^{13}\delta_{me})/35 \quad (1)$$

Although the methyl group derived from the S-adenosylmethionine pool may be somewhat depleted in ^{13}C (Weilacher et al. 1996), it should not significantly affect the overall isotopic composition, because this carbon contributes only 2.8% (1/35) to the chlorophyll *a* nucleus. In all of the chlorophylls and bacteriochlorophylls, eight carbon atoms from positions C−1 through C−4 in glutamic acid are introduced, whereas only one or two carbons from the C−5 position in glutamic acid are incorporated.

In the plastids of photoautotrophic cells, L-glutamic acid is produced by transfer of the amide group of L-glutamine to 2-oxoglutarate, an important metabolite in the citric acid cycle (i.e., tricarboxylic acid cycle or TCA cycle), catalyzed by the enzyme glutamate



Chlorophyll *a*

Fig. 4. Relationships between the carbon positions in glutamate, 5-aminolevulinate (ALA), and chlorophyll *a*. Pathways of glutamate biosynthesis via the citric acid cycle are also shown (modified from Ohkouchi et al. 2008).

synthase as part of the GS-GOGAT cycle (Coruzzi and Last 2000). In unusually nitrogen-enriched environments (>100 mM NH_3), glutamic acid is also synthesized directly from 2-oxoglutarate by incorporation of ammonium by the enzyme glutamate dehydrogenase. In both cases, the carbon skeleton of glutamic acid is inherited from 2-oxoglutarate.

The carbon atoms at the C−4 and C−5 positions in glutamic acid originate from the

methyl and carboxyl groups of acetyl-CoA, respectively. In the citric acid cycle, acetyl-CoA condenses with the four-carbon compound oxaloacetate to form citric acid (Fig. 4; Hayes 2001). 2-Oxoglutarate is formed from citrate by decarboxylation, which does not involve the carbons that originated from acetyl-CoA introduced immediately prior to this step (Fig. 4). Previous studies have indicated that the carboxyl group of acetyl-CoA is substantially depleted in ^{13}C relative to the methyl group due to isotopic fractionation associated with oxidation of pyruvate to acetyl-CoA by the pyruvate dehydrogenase complex (O'Leary 1976; DeNiro and Epstein 1977; Monson and Hayes 1982; Hayes 1993).

The relatively small contribution of the carbon at the C−5 position in glutamic acid to the chlorophyll nucleus could result in its isotopic composition being somewhat enriched in ^{13}C relative to the mean isotopic composition of glutamic acid. Because the isotopic fractionation associated with oxidation of pyruvate to acetyl-CoA is purely a kinetic process, the magnitude of the ^{13}C depletion in the carboxyl carbon may vary widely (Monson and Hayes 1982; Melzer and Schmidt 1987). In case of a 10‰ ^{13}C-depletion of the carboxyl group relative to the methyl group of acetyl-CoA, the chlorophyll nucleus can be calculated to be ~0.6‰ enriched in ^{13}C relative to glutamic acid (or ALA).

In the citric acid cycle of photoautotrophic cells, not only acetyl-CoA but also oxaloacetate derived from irreversible β-carboxylation of phosphoenolpyruvate (PEP) substantially compensate for the metabolites removed from the citric acid cycle (Fig. 4; Sakata et al. 1997). In β-carboxylation of PEP, a bicarbonate carbon (HCO_3^-) binds to the C−4 position of oxaloacetate (corresponding to the C−1 position of 2-oxoglutarate). Typically, HCO_3^- is enriched relative to CO_2 dissolved in seawater by ~10.5‰ at 10° C and ~13.3‰ at 25°C at equilibrium (Mook et al. 1974). Therefore, the carbon isotopic composition of the C−1 position of glutamic acid (i.e., the carboxyl group carbon) should be *significantly* enriched in ^{13}C if β-carboxylation is an important process in the photoautotrophic cell.

In their seminal paper, Abelson and Hoering (1961) reported for the first time the isotopic composition of the C−1 carbon in glutamic acid in photosynthetic microorganisms cultured in the laboratory. They found strong enrichment in ^{13}C (+ 10.9 to + 32.2‰), which was highly variable relative to the mean isotopic composition of the other carbon atoms in glutamic acid (i.e., C−2 through C−5), although their experiments were less precise and based on less carefully cultured organisms than current methods. The above theoretical considerations and experimental observations suggest that the isotopic composition of the methine bridge carbons (i.e., at the C−5, C−10, C−15, and C−20 positions in the tetrapyrrole) are enriched in ^{13}C relative to the mean carbon isotopic composition of the entire chlorophyll nucleus, because they originate from the C−1 carbon in glutamic acid.

Geoporphyrins, geological derivatives of chlorophylls

Because chlorophylls are degraded either chemically or microbiologically, various

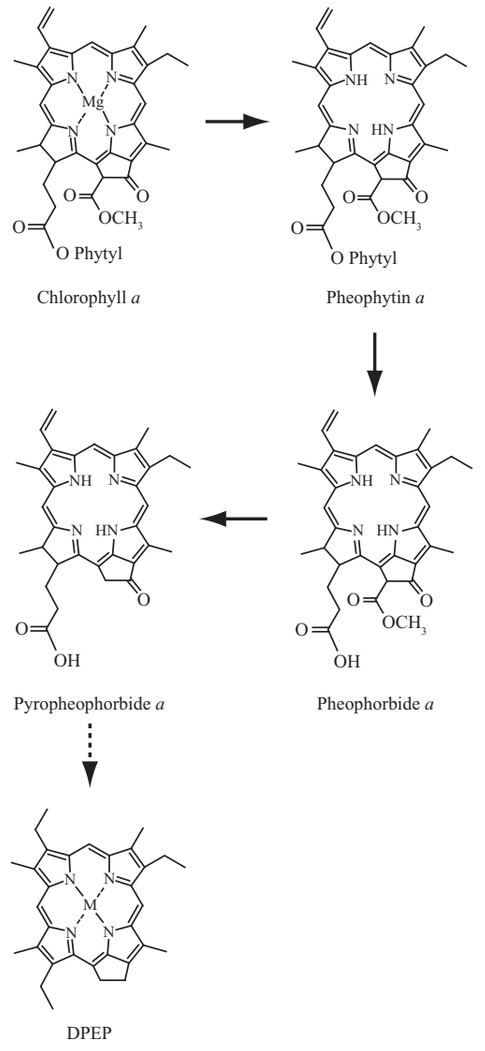


Fig. 5. Degradation (i.e., diagenetic) pathway of chlorophyll *a* in the water column and sediments.

chlorophyll derivatives have been widely observed in the water column and sediments. They include pheophytins which lack the magnesium ion in the center of the tetrapyrrole nucleus of chlorophylls (Fig. 5). Furthermore, pyropheophytins and pyropheophorbides have been reported in many sediments (Fig. 5, e.g., Villanueva et al. 1994; Harris et al.

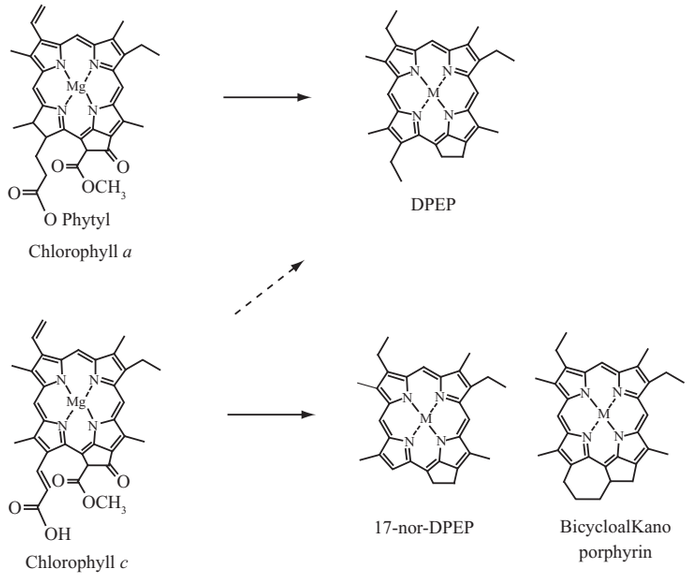


Fig. 6. Source-product relationships between chlorophylls and geoporphyrins.

1995; Nakajima et al. 2003). These "biological tetrapyrroles" are ultimately transformed to geoporphyrins, metallo-porphyrins substituted with various alkyl chains (Baker and Louda 1986; Eckardt et al. 1989; Keely et al. 1990; Kashiyama et al. 2010).

Geoporphyrins are "geological derivatives" of cellular tetrapyrroles including chlorophylls and hemes, and are sometimes termed either sedimentary porphyrins or petroporphyrins. Their origins were first postulated in the 1930s by Alfred Treibs from Hans Fischer's Munich school, who isolated deoxophylloerythroetioporphyrin (DPEP) and etioporphyrin (ETIO; Fig. 6) from a wide range of petroleum, shales, bitumens, and coal. Based on structural similarities, Treibs (1934, 1936) suggested that the DPEP and

ETIO porphyrins originated from chlorophylls and hemes, respectively. Since then, the nomenclature, chemistry, synthesis, and distribution of geoporphyrins in nature have been summarized by several authors (Baker and Palmer 1978; Baker and Louda 1986; Callot and Ocampo 2000; Keely 2006). The purpose of this section is not to thoroughly review geoporphyrins but, rather, to give the readers some insight into the structure and nomenclature of geoporphyrins.

Since the Treibs hypothesis was proposed more than 70 years ago, over 70 species of geoporphyrins have been reported in various sediments, oils, and coals (Callot and Ocampo 2000). Sedimentary porphyrins occur mainly as VO(II) and Ni(II) complexes, although other metal complexes such as Cu(II) and Fe(III) have been frequently observed (Eckardt et al. 1989).

The structural variation in sedimentary porphyrins is far greater than in the original chlorophylls and hemes. In most geological samples, DPEP is most abundant (Baker et al. 1968). Because its exocyclic ring structure is closely related to the chlorophylls, it is generally accepted that DPEP must derive primarily from chlorophylls. Theoretically, all chlorophylls *a*-*d* and even bacteriochlorophyll *a* are potential precursors of sedimentary DPEP. However, it is likely that chlorophyll *a* is the dominant precursor, because it is far more abundant and widespread relative to other chlorophylls in the natural aquatic environment. In contrast, 17-nor-DPEP, a DPEP-type porphyrin lacking an alkyl chain at the C−17 position (Fig. 6), was postulated to be a derivative of chlorophyll *c*, because cleavage of the vinyl group at the C−17 position tends to occur after decarboxylation of the free acrylic chain (Callot et al. 1990). 17-nor-DPEP is widely distributed in geological samples (e.g., Huseby et al. 1996).

Isotopic composition of methine bridge carbon as an indicator of β-carboxylation

The above theoretical considerations suggest that the methine-bridge carbons in chlorophylls should be enriched in ^{13}C relative to the other carbons in proportion to the β-carboxylase activity in the photoautotrophic cell. In other words, the isotopomeric (i.e., intramolecular or site-specific isotopic composition) information on these porphyrins potentially provides physiological information on photoautotrophs in the geological past.

In the present study, we conducted chemical degradation of tetrapyrroles with chromium oxide (Quirke et al. 1980; Grice et al. 1996; Chikaraishi et al. 2008) to investigate their intramolecular isotopic compositions. Chromic acid oxidation of porphyrins produces several types of maleimides (1*H*-pyrrole−2,5-diones) as well as carbon dioxide from the methine bridge carbons (Fig. 7; Chikaraishi et al. 2008). Therefore, the mean isotopic compositions of the four methine bridge carbons of the porphyrins can be estimated using the following mass balance equation, including the isotopic compositions of the bulk tetrapyrroles and the maleimides, the oxidation products of the tetrapyrroles:

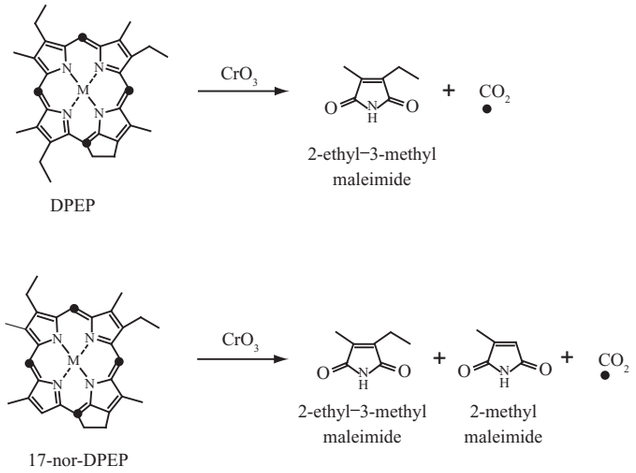


Fig. 7. Chromic acid degradation of DPEP and 17-nor-DPEP to form maleimides.

$$^{13}\delta_{methine} = (32\,^{13}\delta_{porphyrin} - 28\,^{13}\delta_{maleimide})/4$$
$$= 8\,^{13}\delta_{porphyrin} - 7\,^{13}\delta_{maleimide} \quad (2)$$

We evaluated whether isotopic fractionation is associated with chromic acid oxidation in the laboratory experiments. However, we did not observe variations in the estimated mean isotopic compositions of the methine bridge carbons with the maleimide yield of the chromic acid oxidation experiments. Although a more rigorous evaluation is required in the future, based on this evidence, we tentatively concluded that the isotopic fractionation associated with C-C bond cleavage during chromium oxide degradation of tetrapyrrole structures can be neglected.

We applied the above approach to sedimentary porphyrins isolated from the Onnagawa Formation, which was formed in the middle Miocene in a semi-enclosed basin and is distributed in northeast Japan. The Onnagawa Formation mainly consists of diatomaceous deposits with a total organic carbon content of up to 5% by weight; detailed lithology and stratigraphy are described in Tada (1991). From our rock collection, we selected two samples (BA138a and BA138b) for this study (Kashiyama et al. 2008b).

Detailed analytical procedures are described in Kashiyama et al. (2007a) and Chikaraishi et al. (2008). Briefly, the sediments were Soxhlet-extracted with chloroform/methanol (70: 30, v/v). The total extracts were separated with silica gel column chromatography to isolate vanadyl (VO)-porphyrins. Using a two-step HPLC method, we carefully isolated and purified VO-DPEP and VO−17-nor-DPEP from the VO-porphyrins using a computer-controlled fraction collector. Structural assignments for these purified porphyrins were achieved using a combination of absorption spectrometry with a photodiode array detector,

Table 2 Carbon isotopic compositions (‰) of bulk porphyrins from two sediment samples from Onnagawa Formation and maleimides produced by their chromic acid oxidation. Estimated carbon isotopic compositions of methine bridge carbon are also shown in the bottom of the table.

	BA138a		BA138b
	VO DPEP	VO 17-nor-DPEP	VO DPEP
Porphyrin	− 17.1	− 16.7	− 17.9
2-Eethyl−3-methyl-maleimide	− 16.4	− 16.4	− 17.1
2-Methyl-maleimide	n.d.	− 15.7	n.d.
Methine bridge carbon	− 22.0	− 22.3	− 23.5

molecular ion monitoring by single-stage quadrupole mass spectrometry, and single-crystal X-ray diffraction patterns (Kashiyama et al. 2007b, 2008a). Carbon isotopic compositions of individual bulk porphyrins were determined using an elemental analyzer/isotope ratio mass spectrometry (EA/IRMS) with improved sensitivity (Ohkouchi et al. 2005; Ogawa et al. 2010). We have previously reported the detailed carbon isotopic compositions of VO-DPEP and VO−17-nor-DPEP for these samples in Kashiyama et al. (2008b) and they are also shown in Table 2.

We conducted chromic acid oxidation on aliquots of these purified porphyrins to form maleimides. The structural assignments of the maleimides produced by chromic acid oxidation were achieved by gas chromatography/mass spectrometry and comparison with synthetic maleimide standards (GC/MS; Chikaraishi et al. 2008). Carbon isotopic compositions of individual maleimides were determined using gas chromatography/combustion/isotope ratio mass spectrometry (GC/C/IRMS; Chikaraishi et al. 2008).

After chemical degradation of VO-DPEP, we observed 2-ethyl−3-methyl maleimide, a degradation product of rings A, B, and D of the tetrapyrrole structure (Fig. 7). In contrast, chromic acid degradation of VO−17-nor-DPEP produced both 2-ethyl−3-methyl maleimide and 2-methyl maleimide; the former is a product of rings A and B, and the latter is a product of ring D of the tetrapyrrole structure (Fig. 7). The measured carbon isotopic composition of 2-ethyl−3-methyl maleimide from VO-DPEP was −16.4‰ for sample BA138a and −17.1‰ for sample BA138b. Furthermore, those of 2-ethyl−3-methyl maleimide and 2-methyl maleimide from VO−17-nor-DPEP were −16.4‰ and −15.7‰, respectively, for sample BA138a. We were not able to conduct maleimide analysis for VO−17-nor-DPEP from BA138b due to insufficient sample.

Using equation 2 above, we estimated the mean isotopic composition of the methine bridge carbons for these porphyrins. As shown in Table 2, the mean isotopic composition of the methine bridge carbons was −22.0‰ for VO-DPEP and −22.3‰ for VO−17-nor-DPEP from BA138a, whereas that of VO-DPEP from BA138b was −23.5‰. The uncertainty of these estimated isotopic numbers derives not only from instrumental analytical errors (normally ~0.3‰ (1σ) for EA/IRMS and ~0.5‰ (1σ) for GC/C/IRMS), but also from

propagation of these errors. Nevertheless, the notable ^{13}C enrichment of the methine-bridge carbons should be robust, and we conclude that the methine bridge carbons were substantially (5−6‰) depleted in ^{13}C relative to the mean bulk isotopic composition of the original porphyrins.

This isotopomeric evidence clearly suggests that β-carboxylation was not a significant physiological process for photoautotrophs during deposition of the Onnagawa Formation. It is well known that β-carboxylation is a widespread process in C4 and CAM plants (e.g., Hatch and Slack 1970). PEP carboxylase, an enzyme catalyzing this process, has also been widely observed in other organisms including cyanobacteria. Although it is still uncertain which factor controls β-carboxylation activity, 10−20% of the carbon in the cyanobacterial cell has been estimated to be assimilated through β-carboxylation (e.g., Coleman and Colman 1981; Owttrim and Colman 1986). Therefore, our isotopomeric observations in this study seem to be inconsistent with previous results (Kashiyama et al. 2008b). Kashiyama et al. (2008b) estimated an average $\delta^{15}N$ value for the entire photoautotrophic community of −2 to +1‰ based on $\delta^{15}N$ values for DPEP (−6.9 to −3.6‰, n = 7), considering empirical isotopic data indicating that the tetrapyrrole nuclei of chloropigments are depleted in ^{15}N by ~5‰ relative to the cells. This finding suggests that N_2-fixing cyanobacteria were important primary producers during the deposition of the Onnagawa Formation. Alternatively, as also pointed out by Kashiyama et al. (2008b), endosymbiosis between diatoms and cyanobacteria may be a mechanism that explains not only the isotopomeric evidence reported here but also the carbon and nitrogen isotopic compositions of the individual (bulk) porphyrins from these sediments.

Concluding Remarks

We investigated the intramolecular isotopic compositions of tetrapyrrole nuclei of chlorophylls with an emphasis on current knowledge of metabolic processes occurring in photoautotrophic cells. Because their chemical structures, synthetic pathways, and regulatory processes have been intensively studied, chlorophylls are ideal candidates for understanding the carbon cycle in surface water environments. Fortunately, geoporphyrins, geological products of chlorophyll nuclei, are well preserved in the sedimentary record, and thus their isotopic signatures can provide robust evidence regarding the surface water environment in the geologic past.

As suggested by this study, there are strikingly different isotopic ratios among carbon positions within a single molecule depending on the metabolic pathway, and thus, substantial information is recorded in intramolecular isotopic signatures. Therefore, intramolecular carbon isotopic compositions, as well as bulk isotopic compositions, are potentially quite useful for investigating the detailed processes associated with carbon cycling in the euphotic zone of the water column on geological timescales. Nevertheless, there still is not enough evidence strongly supporting the mechanistic approach to interpreting intramolecular isotopic compositions due to our current lack of information. In the authors' view, it is required to rigorously evaluate and understand the relationships between metabolic processes and isotopic fingerprinting, that will undoubtedly bring a bright future to the study of isotopomers of organic molecules.

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References

Abelson PH, Hoering TC (1961) Carbon isotope fractionation in formation of amino acids by photosynthetic organisms. Proc Nat Acad Sci USA 47: 623−632

Baker EW, Corwin AH, Klesper E, Wei PE (1968) Deoxophylloerythroetioporphyrin. J Org Chem 33: 3144−3148

Baker EW, Louda JW (1986) Porphyrins in the geological record. In: Biological Markers in Sediments (Johns RB, Ed). Amsterdam: Elsevier. pp 125−225

Baker EW, Palmer SE (1978) Geochemistry of porphyrins. In: Dolphin D (ed) The Porphyrins Vol 1. Academic Press, London, pp 485−551

Beale SI (1993) Biosynthesis of cyanobacterial tetrapyrrole pigments: hemes, chlorophylls, and phycobilins. In: Bryant DA (ed) The Molecular Biology of Cyanobacteria. Kluwer Academic, Netherlands, pp 519−558

Beal SI (1995) Biosynthesis and structures of porphyrins and hemes. In: Blankenship RE, Madigan MT, Bauer CE (eds) Anoxygenic Photosynthetic Bacteria. Kluwer Academic, Netherkands, pp 153−177

Beale SI, Foley T, Dzelzkalns V (1981) δ-Aminolevulinic acid synthase from *Euglena gracilis*. Proc Nat Acad Sci USA 78: 1666−1669

Beal SI, Gough SP, Granick S (1975) The biosynthesis of δ-aminolevulinic acid from the intact carbon skeleton of glutamic acid in greening barley. Proc Natl Acad Sci USA 72: 2719−2723

Beaumont VI, Jahnke LL, DesMarais DJ (2000) Nitrogen isotopic fractionation in the synthesis of photosynthetic pigments in *Rhodobacter capsulatus* and *Anabaena cylindrica*. Org Geochem 31: 1075−1085

Bidigare R, Kennicutt MC II, Keeney-Kennicutt WL (1991) Isolation and purification of chlorophylls *a* and *b* for the determination of stable carbon and nitrogen isotope compositions. Anal Chem 63: 130−133

Blankenship RE (2004) Molecular Mechanics of Photosynthesis, Blackwell, Oxford.

Blumer M (1965) Organic pigments: their long-term fate. Science 149: 722−726

Brockmann H Jr, Lipinski A (1983) Bacteriochlorophyll *g*. A new bacteriochlorophyll from *Heliobacterium chlorum*. Arch Micobiol 136: 17−19

Callot HJ, Ocampo R (2000) Geochemistry of porphyrins. In: Kadish KM, Smith KM, Guilard R (eds) The Porphyrin Handbook Vol 1. Academic Press, London, pp 350−398

Callot HJ, Ocampo R, Albrecht P (1990) Sedimentary porphyrins: correlations with biological precursors. Energy Fuels 4: 635−639

Chicarelli MI, Hayes JM, Popp BN, Eckardt CB, Maxwell JR (1993) Carbon and nitrogen isotopic compositions of alkyl porphyrins from the Triassic Serpiano oil shale. Geochim Cosmochim Acta 57: 1307−1311

Chikaraishi Y, Kashiyama Y, Ogawa NO, Kitazato H, Satoh M, Nomoto S, Ohkouchi N (2008) A compound-specific isotope method for measuring the stable nitrogen isotopic composition of tetrapyrroles. Org Geochem 39: 510−520

Chikaraishi Y, Matsumoto K, Kitazato H, Ohkouchi N (2007) Sources and transformation processes of pheopigments: carbon and hydrogen isotopic evidence from Lake Haruna, Japan. Org Geochem 38: 985−1001

Chikaraishi Y, Matsumoto K, Ogawa NO, Suga H, Kitazato H, Ohkouchi N (2005) Hydrogen, carbon and nitrogen isotopic fractionations during chlorophyll biosynthesis in C3 higher plants. Phytochemistry 66: 911−920

Coleman JR, Colman B (1981) Photosynthetic carbon assimilation in the blue-green alga, *Coccochloris peniocystis*. Plant Cell Environ 4: 285−290

Coruzzi G, Last R (2000) Amino acids. In: Buchanan BB, Gruissem W, Jones RL (eds) Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists. pp 358−410

DeNiro MJ, Epstein S (1977) Mechanism of carbon isotope fractionation associated with lipid synthesis. Science 197: 261−263

Eckardt CB, Keely BJ, Waring JR, Maxwell JR (1991) Preservation of chlorophyll-derived pigments in sedimentary organic matter. Phil Trans R Soc Lond B 333: 339−348

Eckardt CB, Wolf M, Maxwell JR (1989) Iron porphyrins in the Permian Kupferschiefer of the Lower Rhine Basin, NW Germany. Org Geochem 14: 659−666

Geider RJ (1987) Light and temperature dependence of the carbon to chlorophyll *a* ratio in microalgae and cyanobacteria: implications for physiology and growth of phytoplankton. New Phytol 106: 1−34

Glaeser J, Baneras L, Rütters H, Overmann J (2002) Novel bacteriochlorophyll e structures and species-specific variability of pigment composition in green sulfur bacteria. Arch Microbiol 177: 475−485

Grice K, Gibbison R, Atkinson JE, Schwark L, Eckardt CB, Maxwell JR (1996) Maleimides (1H-pyrrole−2,5-diones) as molecular indicators of anoxygenic photosynthesis in ancient water columns. Geochim Cosmochim Acta 60: 3913−3924

Harris PG, Pearce GS, Peakman TM, Maxwell JR (1995) A widespread and abundant chlorophyll transformation product in aquatic environments. Org Geochem 23: 183−187

Hatch MD, Slack CR (1970) Photosynthetic CO_2-fixation pathways. Ann Rev Plant Phys 21: 141−162

Hayes JM (1993) Factors controlling ^{13}C contents of sedimentary organic compounds: principles and evidence. Mar Geol 113: 111−125

Hayes JM (2001) Fractionation of the isotopes of carbon and hydrogen in biosynthetic processes. In: Vally JW, Cole DR (eds) Reviews in Mineralogy and Geochemistry vol 43. The Mineralogical Society of America. pp 225−277

Hayes JM, Freeman KH, Popp BN, Hoham C (1990) Compound-specific isotopic analysis: a novel tool for the reconstruction of ancient biogeochemical processes. In: Durand B, Behar F (eds) Advances in Organic Geochemistry 1989, Pergamon Press, Oxford, pp. 1115−1128

Hayes JM, Takigiku R, Ocampo R, Callot HJ, Albrecht P (1987) Isotopic compositions and probably origins of organic molecules in the Eocene Messel shale. Nature 329: 48−51

Huseby B, Ocampo R, Bauder C, Callot HJ, Rist K, Barth T (1996) Study of the porphyrins released from the Messel oil shale kerogen by hydrous pyrolysis experiments. Org Geochem 24: 691−703

Iida K, Mimura I, Kajiwara M (2002) Evaluation of two biosynthetic pathways to δ-aminolevulinic acid in *Euglena gracilis*. Eur J Biochem 269: 291−297

Kashiyama Y, Kitazato H, Ohkouchi N (2007a) An improved method for isolation and purification of sedimentary porphyrins by high-performance liquid chromatography for compound-specific isotope analysis. J Chromatogr A 1138: 73−83

Kashiyama Y, Miyashita H, Ogawa NO, Chikaraishi Y, Takano Y, Suga H, Toyofuku T, Nomaki H, Kitazato H, Nagata T, Ohkouchi N (2008c) Evidence of global chlorophyll *d*. Science 321: 658

Kashiyama Y, Ogawa NO, Kuroda J, Kitazato H, Ohkouchi N (2008a) Diazotrophic cyanobacteria as the major photoautotrophs during mid-Cretaceous Oceanic Anoxic Events: nitrogen and carbon isotopic evidence of sedimentary porphyrin. Org Geochem 39: 532−549

Kashiyama Y, Ogawa NO, Nomoto, S, Kitazato H, Ohkouchi N (2010) Nitrogen and carbon isotopic compositions of copper, nickel, and vanadyl porphyrins from the Cretaceous OAE black shales. In: Earth, Life, and Isotopes (Ohkouchi N, Tayasu I, Koba K, Eds), pp. 313−335, Kyoto: Kyoto University Press (in this volume)

Kashiyama Y, Ogawa NO, Tada R, Kitazato H, Ohkouchi N (2008b) Biogeochemistry and ecology of photoautotrophs reconstructed from nitrogen and carbon isotopic compositions of vanadyl porphyrins from Miocene siliceous sediments. Biogeosciences 5: 797−816

Kashiyama Y, Shiro M, Tada R, Ohkouchi N (2007b) A novel vanadyl alkylporphyrins from geological samples: a possible derivative of divinylchlorophylls or bacteriochlorophyll *a*? Chem Lett 36: 706−707

Katase T, Wada E (1990) Isolation of chlorophyll *a* in *Microsystis* spp. for determination of stable isotopes of carbon and nitrogen, and variation in Suwa Lake. Bunsekikagaku 39: 451−456 (in Japanese with English abstract)

Keely BJ (2006) Geochemistry of chlorophylls. In: Grimm B, Porra RJ, RudigerW, Scheer H (eds) Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications. Springer, Netherland, pp 535−561

Keely BJ, Prowse WG, Maxwell JR (1990) The Treibs hypothesis: an evaluation based on structural studies. Energy Fuels 4: 628−634

Kikuchi G, Kumar A, Talmage P, Shemin D (1958) The enzymatic synthesis of δ-aminolevulinic acid. J Biol Chem 233: 1214−1219

Madigan MT, Takigiku R, Lee RG, Gest H, Hayes JM (1989) Carbon isotope fractionation by thermophilic phototrophic sulfur bacteria: evidence for autotrophic growth in natural populations. Appl Environ Microbiol 55: 639−644

Manning WM, Strain HH (1943) Chlorophyll d, a green pigment of red algae. J Biol Chem 151: 1−19

Meier-Augestein W (1999) Applied gas chromatography coupled to isotope ratio mass spectrometry. J Chromatogr A 842: 351−371

Meller E, Belkin S, Harel E (1975) The biosynthesis of δ-aminolevulinic acid in greening maize leaves. Phytochem 14: 2399−2402

Melzer E, Schmidt HL (1987) Carbon isotope effects on the pyruvate dehydrogenase reaction and their importance for relative carbon−13 depletion in lipids. J Biol Chem 262: 8159−8164

Miyashita H, Ikemoto H, Kurano N, Adachi K, Chihara M, Miyachi S (1996) Chlorophyll *d* as a major pigment. Nature 383: 402

Monson KD, Hayes JM (1982) Carbon isotopic fractionation in the biosynthesis of bacterial fatty acids. Ozonolysis of unsaturated fatty acids as a means of determining the intramolecular distribution of carbon isotopes. Geochim Cosmochim Acta 46: 139−149

Mook WG, Bommerson JC, Staberman WH (1974) Carbon isotope fractionation between dissolved bicarbonate and gaseous carbon dioxide. Earth Planet Sci Lett 22: 169−176

Nakajima Y, Okada H, Oguri K, Suga H, Kitazato H, Koizumi Y, Fukui M, Ohkouchi N (2003) Distribution of chloropigments in suspended particulate matter and benthic microbial mat of a meromictic Lake

Kaiike, Japan. Environ Microbiol 5: 1103−1110
Ogawa NO, Nagata T, Kitazato H, Ohkouchi N (2010) Ultra sensitive elemental analyzer/isotope ratio mass spectrometer for stable nitrogen and carbon isotope analyses. In: Earth, Life, and Isotopes (Ohkouchi N, Tayasu I, Koba K, Eds), pp. 339−353, Kyoto: Kyoto University Press (in this volume)
Ohkouchi N, Kashiyama Y, Kuroda J, Ogawa NO, Kitazato H (2006) The importance of diazotrophic cyanobacteria as primary producers during Cretaceous Oceanic Anoxic Event 2. Biogeosciences 3: 467−478
Ohkouchi N, Nakajima Y, Ogawa NO, Suga H, Sakai S, Kitazato H (2008) Carbon isotopic composition of tetrapyrrole nucleus in chloropigments from a saline meromictic lake: a mechanistic view for interpreting isotopic signature of alkyl porphyrins in geological samples. Org Geochem 39: 521−531
Ohkouchi N, Nakajima Y, Okada H, Ogawa NO, Suga H, Oguri K, Kitazato H (2005) Biogeochemical processes in a meromictic lake Kaiike: implications from carbon and nitrogen isotopic compositions of photosynthetic pigments. Environ Microbiol 7: 1009−1016
O'Leary MH (1976) Isotope effect on the enzymatic decarboxylation of pyruvate acid. Biochem Biophys Res Comm 73: 614−618
Owttrim GW, Colman B (1986) Purification and characterization of phosphoenolpyruvate carboxylase from a cyanobacterium. J Bacteriol 168: 207−212
Popp BN, Takigiku R, Hayes JM, Louda JW, Baker EW (1989) The post-Paleozoic chronology and mechanism of ^{13}C depletion in primary marine organic matter. Am J Sci 289: 436−454
Porra RJ (1997) Recent progress in porphyrin and chlorophyll biosynthesis. Photochem Photobiol 65: 492−516
Quirke JME, Shaw GJ, Soper PD, Maxwell JR (1980) Petroporphyrins-II. The presence of porphyrins with extended alkyl substituents. Tetrahedron 36: 3261−3267
Rai AN, Söderbäck E, Bergman B (2000) Cyanobacterium-plant symbioses. New Phytol 147: 449−481
Sachs JP, Repeta DJ, Goericke R (1999) Nitrogen and carbon isotopic ratios of chlorophyll from marine phytoplankton. Geochim Cosmochim Acta 65: 1431−1441
Sakata S, Hayes JH, McTaggart AR, Evans RA, Leckrone KJ, Togasaki RK (1997) Carbon isotopic fractionation associated with lipid biosynthesis by a cyanobacterium: relevance for interpretation of biomarker records. Geochim Cosmochim Acta 61: 5379−5389
Sato T, Miyajima T, Ogawa H, Umezawa Y, Koike I (2006) Temporal variability of stable carbon and nitrogen isotopic composition of size-fractionated particulate organic matter in the hypertrophic Sumida River Estuary of Tokyo Bay, Japan. Est Coast Shelf Sci 68: 245−258
Senge MO, Smith KM (1995) Biosynthesis and structure of the bacteriochlorophylls, In: Blankenship RE, Madigan MT, Bauer C (eds) Anoxygenic Photosynthetic Bacteria. Kluwer Academic Publishers, Netherlands, pp 137−151
Shemin D, Rittenberg D (1946) The life span of the red blood cell. J Biol Chem 166: 627−636
Tada R (1991) Origin of rhythmical bedding in middle Miocene siliceous rocks of the Onnagawa Formation, northeastern Japan. J Sed Petrol 61: 1123−1145
Treibs A (1934) Ueber das Vorkommen von Chlorophyllderivaten in einem Oelschiefer der oberen Trias. Liebigs Ann 509: 103−114
Treibs A (1936) Chlorophyll and hemin derivatives in organic materials. Ang Chem 49: 682−686
Villanueva J, Grimalt JO, de Wit R, Keele B, Maxwell JR (1994) Chlorophyll and carotenoid pigments in solar salten microbial mats. Geochim Cosmochim Acta 58: 4703−4715.
Weilacher T, Gleixner G, Schmidt HL (1996) Carbon isotope pattern in purine alkaloids: a key to isotope discriminations in C1 compounds. Phytochemistry 41: 1073−1077
Zapata M, Garrido JL, Jeffrey SW (2006) Chlorophyll *c* pigments: current status. In: Grimm B, Porra RJ, Rudiger W, Scheer H (eds) Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications. Springer, Netherland, pp 39−53