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Carbon isotopic composition of the tetrapyrrole nucleus in chloropigments from a saline meromictic lake: A mechanistic view for interpreting the isotopic signature of alkyl porphyrins in geological samples

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Abstract

We report carbon isotopic compositions of tetrapyrrole nuclei (chlorophyllides) and side chains (phytol or farnesol) of chlorophyll *a*, bacteriochlorophyll *a*, and bacteriochlorophylls *e* isolated from several depths in the saline meromictic Lake Kaiike, Japan. The chlorophyllides are substantially enriched in ^{13}C relative to the side chains in all the depths of the lake, namely oxic mixolimnion, chemocline, anoxic monimolimnion, and benthic microbial mat. The isotopic compositions of chlorophyllide moieties are strongly related to those of the whole molecule (chloropigments; $r^2 = 0.94$) with the former being $1.8 \pm 0.8‰$ (1σ, $n = 18$) enriched in ^{13}C relative to the latter. No significant difference was observed between chlorophyll species. The heterogeneity of ^{13}C in the chloropigments can be ascribed to differences between the biosynthetic pathways of chlorophyllides and side chains. The alkyl porphyrins in the geological samples are derived from the chlorophyllides, whose carbon skeleton is ultimately inherited from glutamate, one of the major amino acids in the cell. Together with the isotopic relationship between chlorophylls and cell previously reported, we estimate that the carbon isotopic composition of DPEP, a major C_{32} alkyl porphyrin in the geological samples, is ~1.8‰ enriched in ^{13}C relative to the cell.

1. Introduction

The tetrapyrrole nucleus of chloropigments has been known to preserve in sediments and sedimentary rock for long durations of time (*e.g.*, Treibs, 1936; Blumer, 1965; Baker and Louda, 1986; Callot and Ocampo, 2000). Alkyl porphyrins in sediments are often referred to as geoporphyrins, which are derived from the nucleus of chlorophylls. Geoporphyrins have occasionally been found in oil shale and crude oil, suggesting that they are highly resistant to severe pressure/temperature conditions. The molecular distribution of geoporphyrins provides information about photoautotrophic

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communities, and potentially surface water environments in the geological past (*e.g.*, Schaeffer et al., 1993; Keely et al., 1994; Mawson et al., 2004). Furthermore, stable isotopic compositions of carbon and nitrogen in sedimentary porphyrins and chlorins provide information about past biogeochemical processes (Park and Dunning, 1961; Hayes et al., 1987, 1990; Popp et al., 1989; Boreham et al., 1990; Chicarelli et al., 1993; Sachs and Repeta, 1999; Ohkouchi et al., 2006; Kashiyama et al., in press). Degradation products of geoporphyrins (*i.e.*, maleimides) have also been studied successfully for the same purpose (Grice et al., 1996a,b). Since the isotope signatures of the geoporphyrins are long preserved in the sediments, we have enough reason to believe that the intramolecular isotopic signature is also preserved in geoporphyrins.

In the present study, we report carbon isotopic compositions of chloropigments including chlorophyll *a*, bacteriochlorophyll *a*, and bacteriochlorophylls *e* isolated from a modern saline meromictic Lake Kaiike, Japan. The compound-specific isotopic compositions of chloropigments have been previously discussed (Ohkouchi et al., 2005). In this study we focus on the intramolecular carbon isotopic distribution, namely the isotopic compositions of chlorophyllides and the side chains of chloropigments, with an emphasis on the biosynthetic processes of the photoautotrophic cell. This work provides crucial information for the interpretation of carbon isotopic compositions of geoporphyrins in the sediment.

2. Experimental

2.1. Samples

Lake Kaiike is a saline meromictic lake located along the northern coast of Kamikoshiki Island, southwest Japan (Matsuyama, 1977; Nakajima et al., 2003). It is characterized by the permanent existence of an O_2–H_2S interface (*i.e.*, chemocline) around 5 m depth, where dense populations of photosynthetic bacteria, cyanobacteria, and chemosynthetic bacteria form a "bacterial plate" throughout the year (Matsuyama, 1988; Nakajima et al., 2003). Water samples were collected from several depths, including the chemocline at 5 m depth and filtered through a Whatman GF/F filter (0.7 μm pore size) to collect suspended particulate matter. The filters were quickly frozen by dry ice in the field and stored at −30 °C in the laboratory until analysis. Benthic microbial mat sample was recovered near the deepest site of the lake (10.3 m depth) by a SCUBA diver using a plastic corer (10 cm in diameter and about 30 cm long). The core was sliced at 1 cm intervals and stored at −30 °C until analysis.

2.2. Analytical procedures

Detailed procedures for the extraction, separation, and purification of chloropigments are described in Ohkouchi et al. (2005). Briefly, the suspended particulate matter and microbial mat (surface 1 cm) were extracted with acetone. Purification of the chloropigments was achieved by a two-step preparative high-performance liquid chromatography (HPLC) method. The first step was rough purification by using normal-phase HPLC and the second step was by reversed-phase HPLC. During these preparative HPLCs, great care was taken to collect the entire peaks of target compounds to avoid isotopic fractionation (Bidigare et al., 1991). Carbon isotopic measurements were performed with a ThermoFinnigan Delta Plus XP isotope-ratio mass spectrometer coupled to a Flash EA1112 Automatic Elemental Analyzer *via* a Conflo III interface. The isotopic composition was expressed in conventional δ notation against Vienna PeeDee Belemnite (VPDB) standard and reported in Ohkouchi et al. (2005). The precision of this measurement, determined by parallel analyses of our laboratory standards, L-alanine (-26.41 ± 0.04‰) and L-proline (-10.08 ± 0.05‰), was 0.08‰.

Aliquots of the purified chloropigments were hydrolyzed with 0.5 M KOH/MeOH for 2 h under reflux. In this process, the chloropigments were transformed according to the following equations (see also Appendix);

Chlorophyll *a*
→ Chlorophyllide *a* + phytol + MeOH

Bacteriochlorophyll *a*
→ Bacteriochlorophyllide *a* + phytol + MeOH

Bacteriochlorophylls *e*
→ Bacteriochlorophyllides *e* + farnesol

where the chlorophyllide is a heteroaromatic ring with alkyl substituents and functional groups. (N.B. Throughout this study, we refer to the structures of chlorophyllide or bacteriochlorophyllide as fatty acids rather than fatty acid methylesters at the

C-13[3] position.) Upon cooling, they were extracted with *n*-hexane. The extracts were subjected to silica gel column chromatography. An alcohol fraction was derivatized as a trimethylsilylether (TMS) by bis-trimethylsilyltrifluoroacetamide (BSTFA).

The carbon isotopic compositions of isoprenoid side chains (phytol and farnesol) were determined using a ThermoFinnigan Delta Plus XP mass spectrometer connected to an Agilent 6890 gas chromatograph (GC) through a Combustion Interface III. The GC column temperature was programmed from 50 to 120 °C at 30 °C min^{-1} and from 120 to 310 °C at 6 °C min^{-1} with a final hold time of 10 min. A DB-1ms column (30 m × 0.32 mm i.d., 0.1 μm film thickness) was used for all analyses. The carbon isotopic composition was described in conventional δ notation relative to the VPDB standard. The contribution of trimethylsilyl carbon during the derivatization was eliminated by isotopic mass balance calculation. Analytical precision (1σ) was generally better than 0.3‰ as determined by the repeated injections of a mixture of *n*-alkane standards.

The carbon isotopic compositions of chlorophyllides were estimated by the mass balance calculations. In this calculation, we assumed that the carbon isotopic composition of the MeOH produced by the saponification of chlorophyll *a* and bacteriochlorophyll *a* is equivalent to those of chlorophyllides, and neglect the effect of the MeOH loss on the calculation of δ values of chlorophyllides. The carbon of MeOH originated from the methyl group of S-adenosylmethionine, which esterifies the propionic acid side chain attached to the C-13 position of Mg protoporphyrin IX (Beale, 1993).

3. Results

The carbon isotopic compositions of the chlorophyllides and side chains were illustrated in Fig. 1 and also listed in Table 1. Both chlorophyllide *a* and phytol (as a side chain of chlorophyll *a*) in the chemocline (5 m depth) are substantially (4.5‰ and 4.1‰, respectively) depleted in ^{13}C relative to those from the oxic mixolimnion (3 m depth). In the mixolimnion, chlorophyll *a* is produced mainly by diatoms (Kashima, 1989). In the bacterial plate, 16S rDNA analysis indicated that the chlorophyll *a* is produced by unicellular cyanobacteria *Synechococcus* sp. (Koizumi et al., 2004). Since dissolved inorganic carbon (DIC) in the chemocline is 4.2‰ depleted in ^{13}C relative to that of the mixolimnion

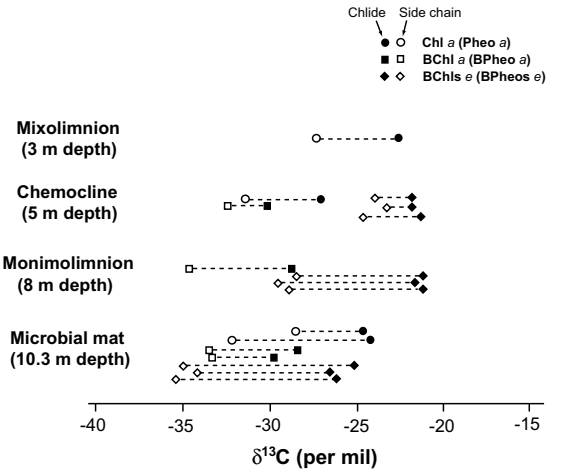

Fig. 1. Carbon isotopic compositions of (bacterio)chlorophyllide and side chains (phytol or farnesol) in chloropigments from various depths in Lake Kaiike, Japan.

(Ohkouchi et al., 2005), the isotopic differences observed in the chlorophyllide *a* and phytol between these depths could be ascribed mostly to that of the substrate, DIC. In the benthic microbial mat, the isotopic compositions of both chlorophyllide *a* and phytol are somewhat between those observed in the mixolimnion and chemocline. This may simply reflect the dual contribution of diatoms and cyanobacteria to the microbial mat.

We observed abundant bacteriochlorophyll *a* (up to 119 μg l^{-1}) produced by purple sulfur bacteria *Halochromatium* sp. in the chemocline (Koizumi et al., 2004; Appendix), where the carbon isotopic compositions of bacteriochlorophyllide *a* and phytol (as a side chain of bacteriochlorophyll *a*) are −30.1‰ and −32.6‰, respectively (Fig. 1). These values are somewhat (1.2‰ and 2.9‰, respectively) lower than those from chlorophyllide *a* and phytol derived from chlorophyll *a* from the same depth. The same trend can be observed in the microbial mat. The biochemical pathways for carbon assimilation and chlorophyll biosynthesis of the purple sulfur bacteria are identical to those of the diatoms and cyanobacteria (Senge and Smith, 1995). Therefore, the cause of the ^{13}C depletion in molecules produced by the purple sulfur bacteria could be ascribed to physiological factors like growth rate, cell size, or geometry (Pancost et al., 1997; Popp et al., 1998; Bidigare et al., 1999).

We also observed abundant (up to 115 μg l^{-1}) bacteriochlorophylls e_1, e_2, and e_3 in the chemocline and anoxic monimolimnion (Nakajima et al., 2003;

Table 1
Summary of isotopic compositions of chloropigments isolated from Lake Kaiike

Sample	Chloropigments	$\delta^{13}C$ (‰)			
		Total	Side chain	Chlorophyllide[a]	Difference
POM (3 m)	Chlorophyll *a*	−24.4	−27.3	−22.7	4.6
POM (5 m)	Chlorophyll *a*	−28.7	−31.4	−27.2	4.2
	Bacteriochlorophyll *a*	−31.0	−32.6	−30.1	2.5
	Bacteriochlorophyll e_1	−22.5	−24.1	−21.8	2.3
	Bacteriochlorophyll e_2	−22.3	−23.5	−21.8	1.7
	Bacteriochlorophyll e_3	−22.3	−24.8	−21.2	3.6
POM (8 m)	Bacteriochlorophyll *a*	−31.0	−34.7	−28.9	5.8
	Bacteriochlorophyll e_1	−23.3	−28.8	−20.9	7.9
	Bacteriochlorophyll e_2	−23.9	−29.7	−21.4	8.3
	Bacteriochlorophyll e_3	−23.4	−29.3	−20.9	8.4
Surface sediment	Chlorophyll *a*	−26.1	−28.4	−24.8	3.6
	Bacteriochlorophyll *a*	−30.3	−33.8	−28.3	5.5
	Bacteriochlorophyll e_1	−28.1	−35.0	−25.1	9.9
	Bacteriochlorophyll e_2	−28.7	−34.4	−26.3	8.1
	Bacteriochlorophyll e_3	−28.8	−35.3	−26.0	9.3
	Pheophytin *a*	−26.8	−32.4	−24.3	8.1
	Bacteriopheophytin *a*	−30.9	−33.5	−29.8	3.7
	Bacteriopheophytin *e*	−25.9	−30.5	−23.9	6.6

[a] Estimated from isotopic compositions of chlorophyll (total) and side chain with mass balance calculation.

Ohkouchi et al., 2005; Appendix). Around the chemocline, they are produced mainly by green sulfur bacteria *Chlorobium phaeovibrioides*, whereas in the anoxic monimolimnion they are mainly produced by a green sulfur bacterium that is genetically close to *Pelodictyon luteolum* (Koizumi et al., 2004). The carbon isotopic relationships between nucleus (bacteriochlorophyllides *e*) and side chains (farnesol) are similar among bacteriochlorophylls e_1, e_2, and e_3 at every measured depth (Fig. 1). However, among different depths, we observed large variations in the isotopic relationships between bacteriochlorophyllides *e* and farnesol. Although the bacteriochlorophyllide *e* homologues have similar isotopic compositions between the chemocline and monimolimnion, the farnesol in the chemocline is substantially (5.2‰ on average) depleted in ^{13}C relative to that of monimolimnion. The ^{13}C depletion of farnesol in the monimolimnion relative to the chemocline could be ascribed to the fact that the green sulfur bacteria (mostly *P. luteolum*) inhabit and photosynthesize using DIC which is substantially depleted in ^{13}C due to regenerated CO_2 in the monimolimnion (Ohkouchi et al., 2005). In the benthic microbial mat, both bacteriochlorophyllides *e* and farnesol are substantially depleted in ^{13}C relative to those in the monimolimnion.

4. Discussion

Fig. 2 illustrates a cross plot of the carbon isotopic compositions of various chlorophyllides and side

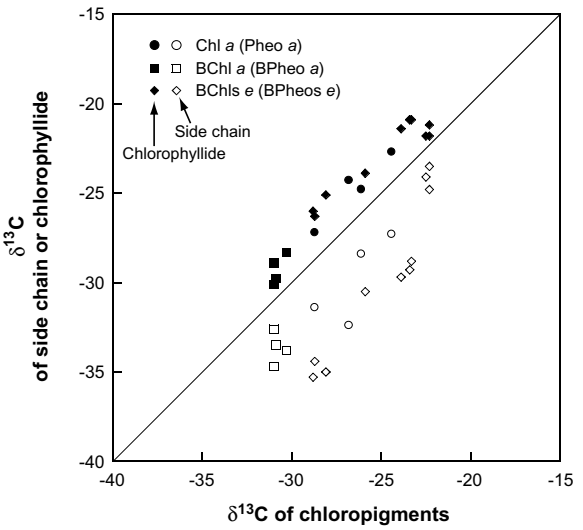

Fig. 2. Carbon isotopic relationships between whole chloropigment molecules and portions of the molecules, chlorophyllides and side chains. Side chains of chlorophyll *a* and bacteriochlorophyll *a* are phytol, whereas those of bacteriochlorophylls *e* are farnesol.

chains (*i.e.*, phytol and farnesol) against those of chloropigments isolated from Lake Kaiike. Clearly, in all chloropigment species, the isotopic compositions of chlorophyllides are strongly related to those of chloropigments ($r^2 = 0.94$) with the former being 0.5–3.0‰ (1.8‰ on average; $1\sigma = 0.8$‰, $n = 18$) enriched in ^{13}C relative to the latter (Fig. 2). Therefore, in this lake the isotopic compositions of chloropigments are related to those of chlorophyllides with some approximation by the following equation:

$$\delta_{\text{chlorophyll}} \approx \delta_{\text{chlide}} - 1.8$$

The subscript and chlide represents chlorophyllides.

Bogacheva et al. (1980) reported that the chlorophyllides of chlorophylls (*a* plus *b*) extracted and purified from fresh nettle leaves are +1.1‰ to +2.7‰ enriched in ^{13}C relative to the whole chlorophyll. More recently, Bidigare et al. (1999) reported that the chlorophyllide is enriched in ^{13}C by 0.9–2.1‰ (1.5‰ on average, $n = 4$) relative to the chlorophylls in suspended particulate matter from the tropical Pacific Ocean. The range of these results is similar to that of Lake Kaiike. However, Sakata et al. (1997) reported that the isotopic difference is somewhat large (3.3‰, $n = 2$) in laboratory cultured cyanobacteria *Synechocystis* sp. Furthermore, in terrestrial C3 higher plants, Chikaraishi et al. (2005) reported that the chlorophyllide is enriched in ^{13}C by 2.3–3.7‰ (3.1‰ on average, $n = 9$) relative to the whole chlorophyll (*a* or *b*). Based on these studies, we can say that it is a common trend that ^{13}C is enriched in the chlorophyllide portion of the chlorophyll.

The ^{13}C enrichment in chlorophyllides relative to the isoprenoid side chains can be reasonably ascribed to the difference of the biosynthetic pathways of these moieties (*e.g.*, Kleinig, 1989; Beale, 1995; Lichtenthaler, 1999). The isoprenoid side chains (*i.e.*, phytol and farnesol) of chloropigments are biosynthesized from isopentenyl diphosphate (IPP) (Kleinig, 1989; Rohmer, 1993), and the carbon isotopic compositions of these side chains are controlled by that of IPP. In cyanobacteria, purple sulfur bacteria, and green sulfur bacteria, the IPP is formed through the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway (Fig. 3; Lichtenthaler, 1999; Boucher and Doolittle, 2000; Lange et al.,

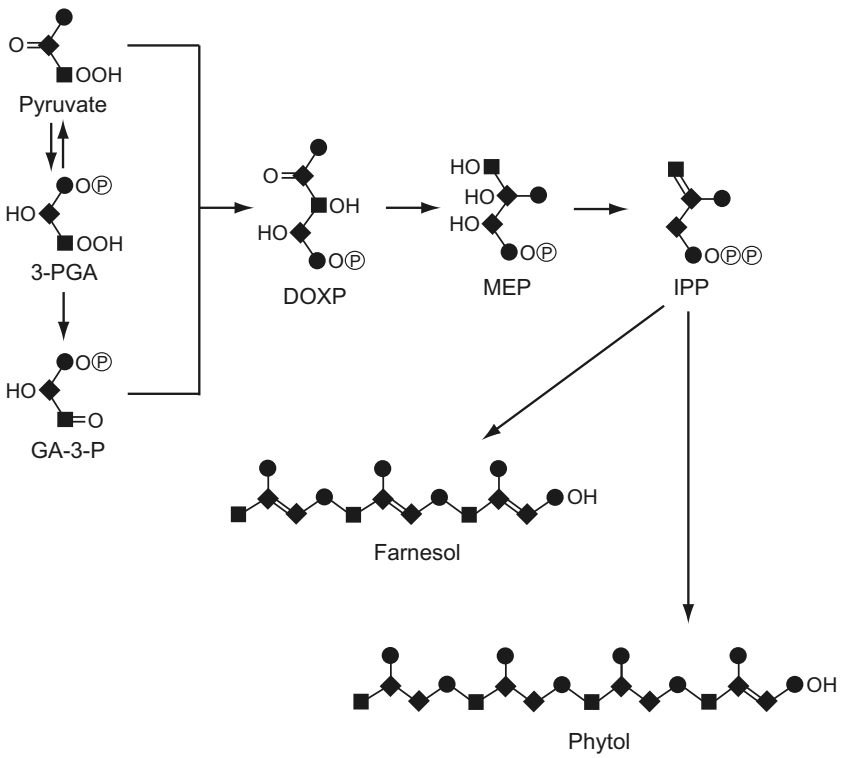

Fig. 3. Biosynthetic pathways of side chains of chloropigments (*i.e.*, farnesol and phytol) and relationships between the carbon positions in these molecules and their sources. Abbreviations: 3-PGA, 3-phosphoglyceric acid; GA-3-P, D-glyceraldehyde-3-phosphate; DOXP, 1-deoxy-D-xylulose-5-phosphate; MEP, 2-C-methyl-D-erythritol-4-phosphate; IPP, isopentenyl diphosphate.

2000). In this pathway, carbons from the C-1, C-2, and C-3 positions of pyruvate contributed to phytol and farnesol through the MEP pathway in a 1:2:2 ratio (Fig. 3; Hayes, 2001; Chikaraishi et al., 2004). Therefore, to understand the carbon isotopic composition of the side chains, we need to know about the isotopic composition of specific carbon positions of pyruvate.

In contrast, the chlorophyllides are produced in plastids by the condensation of eight molecules of 5-aminolevulinate (ALA), which is transformed from glutamate by a multi-enzyme pathway (Fig. 4; *e.g.*, Beale, 1995). Although the α subgroup of purple bacteria produce ALA by the condensation of glycine with succinyl-CoA (Beale, 1993), they were not observed in Lake Kaiike (Koizumi et al., 2004). Based on the experiments with ^{14}C labeled glutamate, it has been demonstrated that the entire carbon skeleton of glutamate is incorporated intact into ALA (Fig. 4; *e.g.*, Beale et al., 1975; Meller et al., 1975).

In the case of 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, which leads the δ value of bacteriochlorophyllide *a* to be the same as

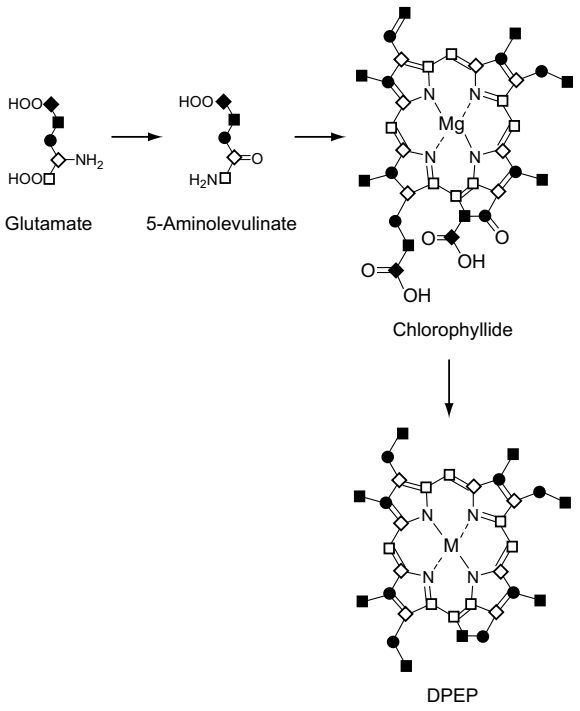

Fig. 4. Relationships between the carbon positions in glutamate, ALA (5-aminolevulinic), chlorophyllide *a*, and DPEP (deoxophylloerythroetioporphyrin).

that of the immediate precursor. In the case of the synthesis of 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 of the bacteriochlorophylls *e* are derived from the methyl group of S-adenosylmethionine, a metabolic product of methionine (Fig. 4, Appendix). Therefore, the carbon isotopic compositions of bacteriochlorophyllides *e* should be slightly modified by this process. In contrast, the synthesis of chlorophyllides does not include this process.

Fig. 4 also illustrates the relationship between the carbon positions in chlorophyllide *a* and its source, glutamate. It should be noted that the five carbon atoms in glutamate are not equally incorporated into the chlorophyllide *a*. Eight carbons derived from each position C-1 through C-4 in glutamate are introduced to the chlorophyllides, whereas only two carbons derived from the C-5 position of glutamate are incorporated into it (Fig. 4). Therefore, in a mechanistic sense, the carbon isotopic compositions of chlorophyllides and bacteriochlorophyllides can be expressed in the following equations:

$$\delta_{\text{chlide } a} = (8\delta_{C-1} + 8\delta_{C-2} + 8\delta_{C-3} + 8\delta_{C-4} + 2\delta_{C-5})/34$$

$$\delta_{\text{bchlide } a} = (8\delta_{C-1} + 8\delta_{C-2} + 8\delta_{C-3} + 8\delta_{C-4} + 2\delta_{C-5})/34$$

$$\delta_{\text{bchlide } e} = (8\delta_{C-1} + 8\delta_{C-2} + 8\delta_{C-3} + 7\delta_{C-4} + \delta_{C-5} + n\delta_{Me})/(32 + n) \quad (n = 2, 3, \text{or } 4)$$

where the subscripts chlide and bchlide represent chlorophyllide and bacteriochlorophyllide, respectively. The *n* and δ_{Me} represent the number and isotopic composition of carbon derived from the methyl group from S-adenosylmethionine. The *n* takes the value from 2 to 4, corresponding to bacteriochlorophylls e_1, e_2, and e_3, respectively. The relative abundances of the added carbon correspond to 5.9–11% of bacteriochlorophyllides. Since we have no information about the carbon isotopic composition of the Me group of S-adenosylmethionine, we do not precisely know how much this affects the overall isotopic compositions, but it might be significant when interpreting their isotope compositions.

In the plastids of photoautotrophic cells, glutamate is primarily produced by the transfer of amide group of glutamine to 2-oxoglutarate (α-ketoglutarate), a metabolite in the citric acid cycle, by the

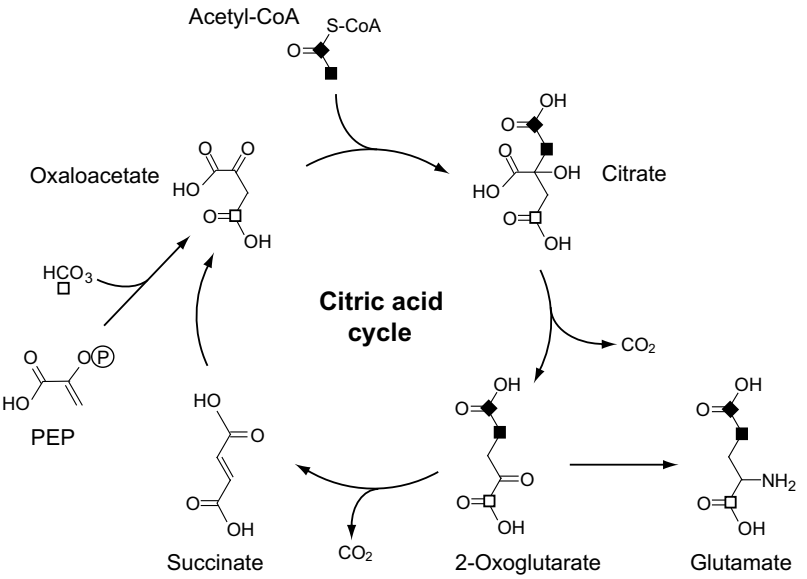

Fig. 5. Pathways of glutamate biosynthesis in the photoautotrophic cell. In the mitochondrion, 2-oxoglutarate is formed as an intermediate of the citric acid cycle. It is transformed to the chloroplast and reacts with glutamine to form two glutamates. Although the citrate is a symmetrical molecule, carbons derived from acetyl-CoA are not randomized, because citrate binds asymmetrically to aconitase, an enzyme catalyzing isomerization of citrate to isocitrate. The two carbons donated by acetyl-CoA can be traced until the first symmetric intermediate succinic acid, at which point they become randomized.

enzyme glutamate synthase (GOGAT; Falkowski and Raven, 1997; Coruzzi and Last, 2000) (Fig. 5). It is also produced directly from 2-oxoglutarate by incorporation of ammonium through the enzyme glutamate dehydrogenase (GDH). In both cases, the carbon skeleton of glutamate originates from 2-oxoglutarate. The carbon atoms at the C-4 and C-5 positions in glutamate originate from methyl and carboxyl groups of acetyl-CoA, respectively, which is condensed with the four-carbon compound oxaloacetate to form citric acid in the citric acid cycle (Fig. 5; Hayes, 2001). 2-Oxoglutarate is formed from citrate by decarboxylation, which is not related to the carbons that originate from acetyl-CoA introduced immediately before this step (Fig. 5). Previous studies indicate that the carboxyl group of acetyl-CoA is substantially depleted in ^{13}C relative to the methyl group due to the isotopic fractionation associated with the oxidation of pyruvate to acetyl-CoA by pyruvate dehydrogenase complex (O'Leary, 1976; DeNiro and Epstein, 1977; Monson and Hayes, 1982; Hayes, 1993). The relatively small contribution of carbon at the C-5 position in glutamate to the chlorophyllide could lead to the isotopic composition of chlorophyllide being somewhat enriched in ^{13}C relative to glutamate. Since the isotopic fractionation associated with the oxidation of pyruvate to acetyl-CoA is purely a kinetic process, the magnitude of the ^{13}C depletion in carboxyl carbon potentially varies widely (Monson and Hayes, 1982; Melzer and Schmidt, 1987). In case that the carboxyl group of acetyl-CoA is 5‰ depleted in ^{13}C relative to the methyl group, the isotopic composition of chlorophyllide is calculated to be approximately 0.3‰ enriched in ^{13}C relative to the glutamate (or ALA).

In the citric acid cycle, the oxaloacetate is derived both from malic acid, a precursor in the cycle and β-carboxylation of phosphoenolpyruvate, which compensates for the metabolites being removed from the cycle. In the β-carboxylation of phosphoenolpyruvate, the ^{13}C enriched carbon from bicarbonate locates at the C-4 position of oxaloacetate (corresponding to the C-1 position of 2-oxoglutarate). Therefore, the carbon isotopic composition of the C-1 position of glutamate should be enriched in ^{13}C. In their classic paper, Abelson and Hoering (1961) determined the isotopic composition of C-1 carbon in glutamate from photosynthetic microorganisms, including purple sulfur bacteria (*Chromatium* sp.) cultured in the laboratory. They reported that the carbon at the C-1 position in glutamate produced by the photoautotrophs is strongly enriched in ^{13}C (+10.9‰ to +32.2‰) but is highly

variable relative to the average of other carbons (*i.e.*, C-2 through C-5). The above theoretical consideration and experimental observation suggest that alkyl maleimides and degradation products of chlorophyllides in the geological samples (Grice et al., 1996a,b) are somewhat depleted in ^{13}C relative to the source chlorophyllides, because the carbons at methine bridge (*i.e.*, C-5, C-10, C-15, and C-20 positions) removed in the degradation process originate from C-1 carbon in glutamate (Fig. 4).

4.1. Implications for paleobiochemical study

Our results provide essential information when interpreting the carbon isotopic record of alkyl porphyrins (as metal complexes) in geological samples. Since these "geoporphyrins" are derived mostly from photoautotrophs (*e.g.*, Baker and Louda, 1986), the carbon isotopic compositions of the geoporphyrins have been used for the reconstruction of photoautotrophic cells (*e.g.*, Hayes et al., 1987; Popp et al., 1989; Kashiyama et al., in press). Such evidence potentially provides high quality information about biogeochemical processes in surface water from the distant past.

In most geological samples, metal complexes of C_{32} deoxophylloerythroetioporphyrin (DPEP; Appendix) have been observed to be the most abundant alkyl porphyrin species (Baker et al., 1968; Baker and Louda, 1986; Callot and Ocampo, 2000). C_{32} DPEP is characterized by a five-membered exocyclic ring, strongly suggesting it to have originated from chlorophylls. During post-depositional alteration, two carbons at the C-13[3] and C-17[3] positions are removed from the chlorophyllide to form C_{32} DPEP (Baker and Louda, 1986; Keely et al., 1990; Eckardt et al., 1991; Callot and Ocampo, 2000). Since these two carbons both originate from the C-5 carbon of glutamate (Fig. 4), the isotopic composition of C_{32} DPEP can be expressed as,

$$\delta_{DPEP} = (8\delta_{c1} + 8\delta_{c2} + 8\delta_{c3} + 8\delta_{c4})/32 = (\delta_{c1} + \delta_{c2} + \delta_{c3} + \delta_{c4})/4$$

This equation indicates that the carbon isotopic composition of C_{32} DPEP is equivalent to the mean isotopic composition of carbon from the C-1 to C-4 positions in glutamate produced by the source photoautotrophs. When compared with chlorophyllide *a* and bacteriochlorophyllide *a*, only two carbons out of 34 (5.9%) are removed during diagenesis. Since the removed carbon atoms are derived from C-5 carbon of glutamate (Fig. 4), this process could lead to a slight enrichment in ^{13}C due to the reasons described above.

Together with C_{32} DPEP, lower-molecular-weight (C_{29}–C_{31}) alkyl porphyrins were generally observed in various geological samples (*e.g.*, Baker and Louda, 1986; Callot and Ocampo, 2000; Kashiyama et al., in press). For example, C_{30} DPEP-type porphyrins have been proposed to be formed by devinylation of either C-3, C-8, or C-17 position of chloropigments (Fookes, 1983; Verne-Mismer et al., 1988; Sandararaman and Boreham, 1991; Kashiyama et al., 2007). The carbon atoms that form ethyl groups at the C-3, C-8, C-13, and C-17 positions of C_{32} DPEP are ultimately derived from carbon atoms at the C-3 and C-4 positions of glutamate (Fig. 4). Thus, we must keep intramolecular isotopic heterogeneity in mind when interpreting the carbon isotopic compositions of lower-molecular-weight geoporphyrins.

5. Conclusions

In previous reports, it has been suggested that chlorophyllides are slightly (~0.5‰) enriched in ^{13}C relative to the cell (Hayes et al., 1987; Laws et al., 1995; Bidigare et al., 1999). Combining the carbon isotopic relationship between porphyrin and chlorophyll discussed in this study, the cellular isotopic composition can be reconstructed with some approximation by the following equation:

$$\delta_{cell} \approx \delta_{DPEP} - 1.8\ (‰)$$

In the modern aquatic environment, oceanographers/geochemists measured the carbon isotopic composition of cells (most cases as particulate organic carbon) or chlorophylls for understanding the carbon cycle. Therefore, the above equation is useful for connecting the porphyrin isotopic data with the paleoenvironment.

However the amplitude of apparent ^{13}C enrichment in the chlorophyllide relative to the chlorophyll or side chain is not well understood. Care must be taken in the mechanistic approach for understanding the isotopic record of geoporphyrins, until we are sufficiently knowledgeable about the intramolecular isotopic distribution of glutamate or pyruvate. Unfortunately, our current knowledge about the intramolecular isotopic distributions of these molecules is very limited, and clearly we need to know more about them. With such information,

we can more precisely discuss the environment of the surface oceans in the geological past.

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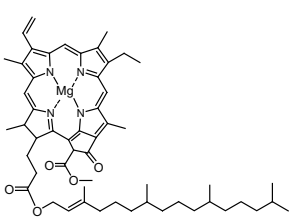
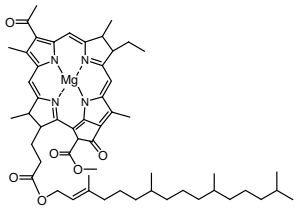
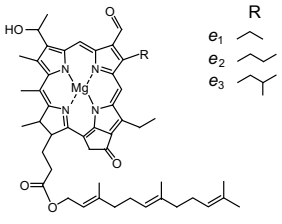
Appendix. Chemical structures of compounds discussed in the text



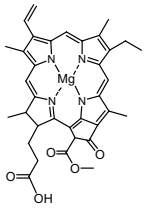
I. Chlorophyll *a*



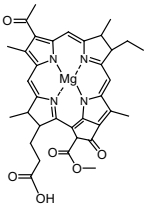
II. Bacteriochlorophyll *a*



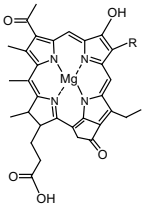
III. Bacteriochlorophyll *e*



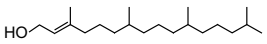
IV. Chlorophyllide *a*



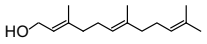
V. Bacteriochlorophyllide *a*



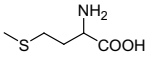
VI. Bacteriochlorophyllide *e*



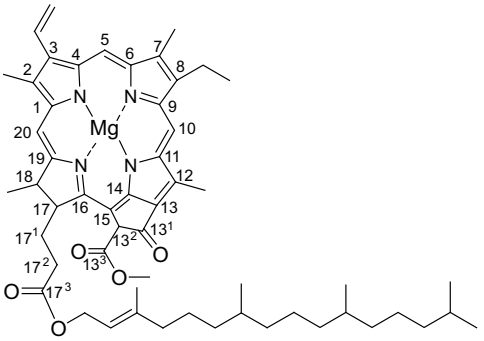
VII. Phytol



VIII. Farnesol



IX. Methionine



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