The First Experimental Simulation of Thermal Transformation of Chlorophylls into Benzoporphyrins in Sediments

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Heating of chlorophyll *a* was performed at 350 °C for 12 h in relation to the origin of sedimentary benzoporphyrins. The product was oxidized by chromic acid to give phthalimide and its methyl homologs, which indicated the formation of benzopyrrole units in the substrate or its degraded fragments. The heating also produced a monomethylpyrrole unit, suggesting that a vinyl group of the chlorophyll was used as a part of the thermally generated benzopyrrole units.

Sediments and petroleum commonly contain porphyrins, which are principally derived from chlorophylls and are usually found as complex mixtures with various substituents on their pyrrole rings due to chemical modification in the sediments.[1–4] Sedimentary porphyrins, therefore, have been appreciated as a biological marker as well as a chemical indicator for sediment maturity. Benzoporphyrins (Chart 1) have been found as minor components of sedimentary porphyrins.[5,6] The first chemical evidence for their presence in a crude oil was given by chromic acid oxidation of isolated porphyrin fractions producing phthalimide and one of its methyl homologs[7] (Chart 1). Later, benzoporphyrins were isolated from a crude oil and their structures were determined by NMR spectroscopy.[8] Benzoporphyrins are evidently diagenetic products because of the absence of porphyrins with this structural feature in plants and animals. Several suggestions have been given as to their formation pathway in sediments,[9–16] among which the Diels-Alder hypothesis seems to be the most likely. This hypothesis proposes [2 + 4] cycloaddition of plastoquinones with a methylvinylpyrrole moiety of divinylchlorophyll *a* (Chart 1) and subsequent aromatization of the resulting cyclohexene ring.[15] However, no experiment under simulated sedimentary conditions has been given to show formation of benzoporphyrins. In the present study, we demonstrated that heating of chlorophyll *a* alone generates a benzopyrrole unit in the molecule, which gives the first experimental evidence in support of diagenetic transformation of chlorophylls into benzoporphyrins.

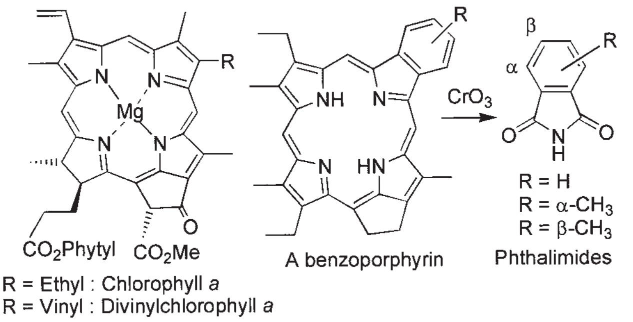
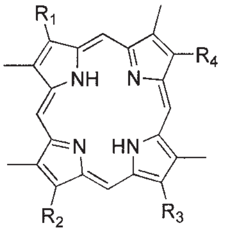


Chart 1.

Commercial chlorophyll *a*, protoporphyrin, and hematin (0.12–0.37 mg, Chart 2) were heated at 350 °C for 12 h in a degassed sealed tube. The product was oxidized with 10% CrO_3 in 25% H_2SO_4 (1 ml) at 0 °C for 2 h and then at room temperature for 2 h. Almost all the procedures were carried out in the dark. The resulting maleimides and phthalimides were extracted with benzene and analyzed by GC-MS. Identification and quantification of the oxidation products were performed by comparison of the GC-MS data with those of authentic samples.[17]



	R_1	R_2	R_3	R_4
Protoporphyrin	V	CE	CE	V
Hematin	Fe^{III}(OH) Protoporphyrin			
Etioporphyrin	E	E	E	E

V : vinyl, CE : 2-carboxyethyl, E : ethyl.

Chart 2.

Figure 1 shows gas chromatograms obtained by the heating experiment. Chromic acid oxidation[18] of unheated chlorophyll *a* yielded only 2-ethyl-3-methylmaleimide and 6,10,14-trimethylpentadecan-2-one (Figure 1a), derived from an ethylmethylpyrrole and a phytyl moiety, respectively. On the other hand, we observed the peaks of phthalimide and α- and β-methylphthalimides in the chromatograms (Figures 1b and 1c) of an oxidation product of chlorophyll *a* heated at 350 °C for 12 h. By this fact was evidenced the formation of a benzopyrrole unit in the chlorophyll

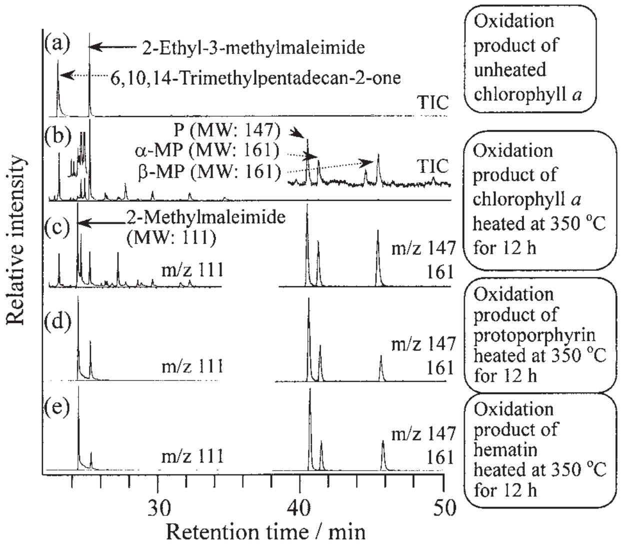


Figure 1. Total ion chromatograms and mass fragmentograms of oxidation products of unheated chlorophyll *a* (a), heated chlorophyll *a* (b and c), heated protopophyrin (d), and heated hematin (e). P: phthalimide, MP: methylphthalimide.

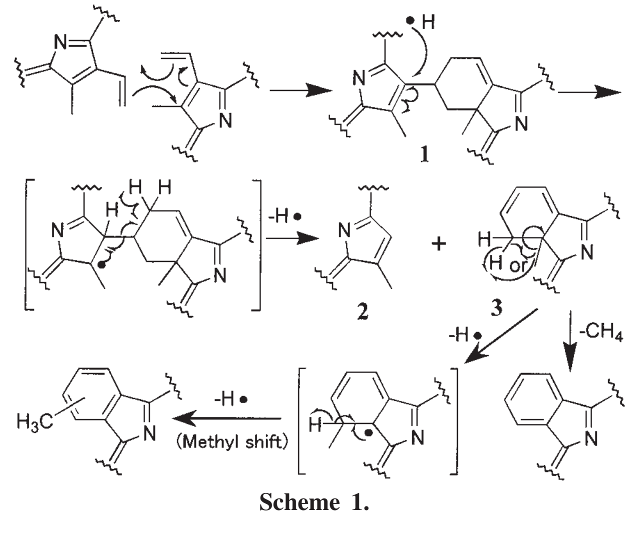
molecule or its pyrolyzed fragments during the heating, although heating seemed to cause degradation or fragmentation of chlorophyll.

Generation of methylbenzopyrrole units from chlorophyll was also revealed by this experiment, which is of significance in view of chlorophyll diagenesis, since we had previously found both α- and β-methylphthalimides together with phthalimide in oxidative extracts of two series of sediments.[19,20] The present results support the suggestion that phthalimide and its methyl homologs, obtained by oxidative extraction from sediments, could be appreciated as novel biomarkers of photosynthesizing organisms.

The total yield of phthalimide and its methyl homologs was 0.36% on the basis of the starting chlorophyll, and the yields of 2-ethyl-3-methyl- and 2,3-dimethylmaleimide were 17 and 3.1%, respectively. The latter maleimide was already shown to be derived from an ethylmethylpyrrole moiety through pyrolytic demethylation in our previous report, in which we showed that the yield of the chromic acid oxidation was 10 to 20%.[21] Therefore, the production of benzopyrrole units should have taken place to an extent several times larger than that observed for the yield of the phthalimides. Heating of protoporphyrin and hematin gave the phthalimides in the total yields of 1.4 and 4.9% (Figures 1d and 1e), respectively.

Heating of chlorophyll *a* also afforded 2-methylmaleimide after oxidation in 2.0% yield (Figure 1c). Since only a negligible amount of this maleimide had been formed in our previous heating experiment using etioporphyrin (Chart 2),[21] the maleimide may be derived from a methylvinylpyrrole moiety of the chlorophyll.

It is likely that a vinyl group of chlorophyll *a* is used as a part of the benzopyrrole units in the thermal chlorophyll transformation. We postulated consequently the mechanism of the benzopyrrole formation as illustrated in Scheme 1: a Diels-Alder adduct (**1**) is formed at first, and its C–C bond bridging two chlorophyll molecules is cleaved in a similar way to the previously proposed mechanism of the devinylation of vinyl-substituted porphyrins through addition of resorcinol to a vinyl group and subsequent elimination of vinylresorcinol,[22] to give a methylpyrrole (**2**) and a dihydrobenzopyrrole unit (**3**). The radical mechanism adequately explains the formation of these pyrrole units from **1** as well as subsequent reactions, since generation of radicals including a hydrogen atom can be assumable in the reaction system at the temperature employed here, as demonstrated in the previously reported pyrolysis of dihydrophytol at 320 °C.[23] The cyclohexadiene part of **3** may be easily aromatized under the heating conditions to generate a benzopyrrole unit. Migration of a bridgehead methyl group of **3** produces α- and β-methylbenzopyrrole units. The scheme shown here may provide a candidate for plausible pathways leading to benzoporphyrin formation.



Scheme 1.

In conclusion, we have provided the first experimental results which can explain diagenetic transformation of chlorophylls into benzoporphyrins without considering the intervention of any other reactant compounds. We also revealed that this transformation accompanies formation of α- and β-methylbenzopyrrole units as well as a monomethylpyrrole unit. Based on these findings, we are able to extend the scope of understanding of chlorophyll diagenesis in sediments.

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