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Biomarker Compositions of Dinoflagellates and Their Applications for Paleoenvironmental Proxies

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ABSTRACT

Four motile cells of autotrophic dinoflagellates, *Peridinium umbonatum var. inaequale*, *Akashiwo sanguinea*, *Scrippsiella tinctoria*, and *Prorocentrum micans*, commonly contain five major sterols: cholesterol, 4-methylcholestan-3-ol, 4, 24-dimethylcholestan-3-ol, dinosterol and dinostanol. A motile cell of heterotrophic dinoflagellate, *Protoperidinium crassipes*, contains cholesterol, 4, 24-dimethylcholestan-3-ol, dinosterol, dinostanol and 4-tetramethylcholestan-3-ol as major free sterols. Dinosterol concentration of heterotrophic dinoflagellate is about 4–12 times higher than those of autotrophic species, suggesting that the heterotrophic dinoflagellate is important source of dinosterol in some sediments. 4-Methylcholestan-3-ol and 4-tetramethylcholestan-3-ol occur respectively in autotrophic and heterotrophic dinoflagellates are believed to serve as potential biomarkers in respective types. Resting cyst of *P. inaequale* contains 4-methylcholestan-3β-ol, 4, 24-dimethyl-5α-cholestan-3β-ol, dinosterol, dinostanol, and unknown C_{31} sterol as major free sterols. The unknown C_{31} sterol detected only in resting cyst could have been produced during resting stage. This compound may serve as a potential biomarker for resting cysts of dinoflagellate.

Keywords: Autotrophic dinoflagellate, Heterotrophic dinoflagellate, Resting cyst, 4α-Methyl sterol

INTRODUCTION

Dinoflagellate is one of the major primary producers in the ocean since the Mesozoic. These microalgae occur throughout the world's oceans but are often more abundant in coastal areas. About half species of dinoflagellate are autotrophic ones, and others are heterotrophic. Some species are able to force themselves into a dormant or resting stage as part of their relatively complicated life cycle. These dormant stages, called resting cysts, are typically characterized by a thick and highly specialized cell covering. The motile stage of dinoflagellate is hardly recorded in sediments since the motile cell of dinoflagellate is labile against bio- and chemical degradations during the settling and the early diagenesis. The resting cysts of some dinoflagellates species are composed of resistant biomacromolecules, which can be preserved in sediments and sedimentary rocks. Geologic record of dinoflagellate evolution, therefore, is based on their resting cyst fossils in sedimentary rocks.

The sterol compositions of dinoflagellates are generally dominated by 4α-methyl sterols including

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the C_{30} sterol called dinosterol (4α, 23, 24-trimethyl-5α-cholest-22*E*-en-3β-ol). This sterol is rarely found in other algae and hence has been often used as an indicator of dinoflagellate contribution to the marine sediments. However, sedimentary dinosterols do not necessarily provide sufficient information about the motile stage of dinoflagellate and the relative contribution of heterotorophic and autotrophic dinoflagellates. Sterol compositions vary quite considerably between different species of dinoflagellates [1]. Some particular sterols can be potential biomarkers for motile cells of dinoflagellates. The objective of the present study is to search for characteristic sterols of motile cell and resting cyst of autotrophic and heterotorophic dinoflagellates.

MATERIALS AND METHODS

Five motile cells of dinoflagellates (*Peridinium umbonatum var. inaequale*, *Akashiwo sanguinea*, *Scrippsiella tinctoria*, *Prorocentrum micans*, *Protoperidinium crassipes*) and a resting cyst of *P. inaequale* were cultured. After the cultivation, the samples were collected at GF/F filters. Lipids were extracted by ultrasonication with methanol/dichloromethane. The combined lipid extract was saponified with 0.5 mol KOH/methanol. The neutral fraction was extracted with diethylether/*n*-hexane (1 : 9), and was fractionated into four fractions by silica gel column chromatography. The sterol fraction was analyzed using a Hewlett Packard 6890 series gas chromatograph (GC). The compounds were identified by mass spectral analysis using a gas chromatograph-mass spectrometer (GC/MS) (Hewlett Packard GC HP6890 and MS HP5973).

RESULTS AND DISCUSSION

Four motile cells of autotrophic dinoflagellates, *Peridinium umbonatum var. inaequale*, *Akashiwo sanguinea*, *Scrippsiella tinctoria*, and *Prorocentrum micans*, commonly contain five major sterols in the free sterol fraction (Fig. 1). These major sterols are cholesterol, 4-methylcholestan-3-ol, 4, 24-dimethylcholestan-3-ol, dinosterol and dinostanol (4, 23, 24-trimethylcholestan-3-ol). A motile cell of heterotrophic dinoflagellate, *Protoperidinium crassipes*, contains cholesterol, 4, 24-dimethylcholestan-3-ol, dinosterol, dinostanol and 4-tetramethylcholestan-3-ol as major free sterols (Fig. 1). The dinosterol concentrations of autotrophic dinoflagellates vary within a range of 0.18 to 0.52 pg/cell. The dinosterol concentration of heterotrophic dinoflagellate *P. crassipes* is 2.3 pg/cell, which is about 4–12 times higher than the range for autotrophic species. The genus *Protoperidinium* currently includes more than 200 spe-

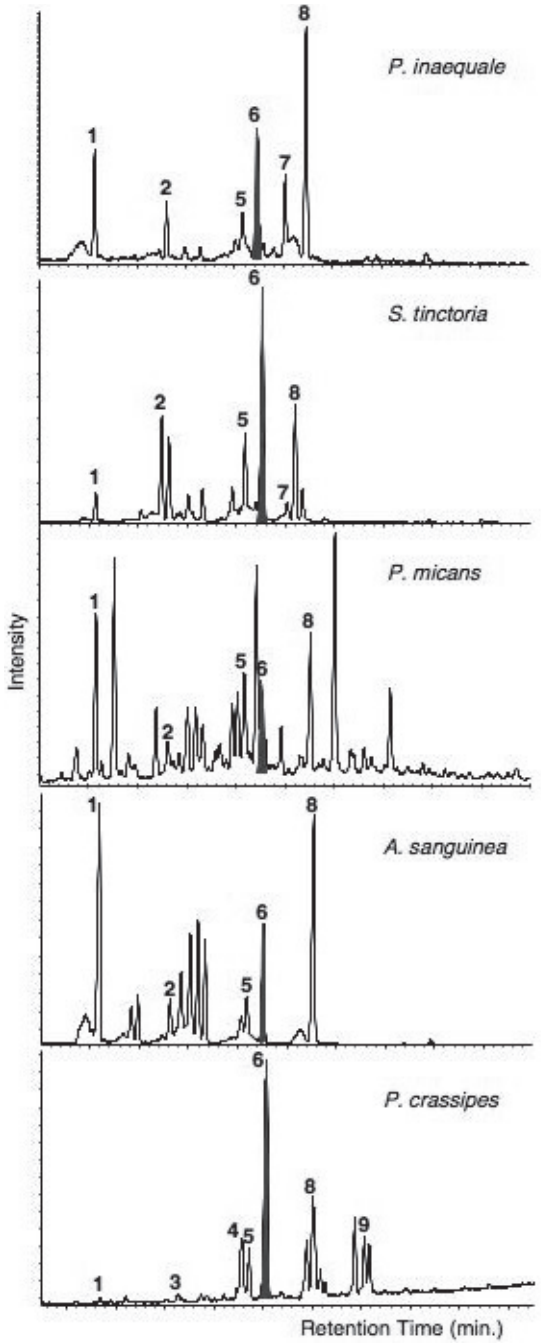

Fig. 1 Total ion chromatograms of sterol fractions from all motile cells of dinoflagellates. 1: Cholesterol, 2: 4*α*-Methylcholestan-3*β*-ol, 3: Campesterol, 4: *β*-Sitosterol, 5: 4*α*, 24-Dimethyl-5*α*-cholestan-3*β*-ol, 6: Dinosterol, 7: 4*α*-23, 24-Trimethylcholest-17 (20)-en3*β*-ol, 8: 4*α*, 23R, 24R-Trimethyl-5*α*-cholestan-3*β*-ol, 9: 4*α*-Tetramethycholestan-3*β*-ol.

cies, and its members are some of the commonest organisms in the neritic plankton [2]. These observations suggest that the heterotrophic dinoflagellate can be an important source of dinosterol in some sediments. The dinosterol flux is not always applicable as the proxy of primary production by dinoflagellates in those sediments, in which heterotrophic dinoflagellates are significant contributors of organic matter.

4-Methylcholestan-3-ol was commonly detected in autotrophic dinoflagellates, but not in heterotrophic dinoflagellate in the present study (Fig. 1). On the contrary, 4-tetramethylcholestan-3-ol was detected only in heterotrophic dinoflagellate (Fig. 1). The sterol composition of dinoflagellates is dominated by 4-methyl sterols, including dinosterol, which is found in many dinoflagellate species [e.g. 3]. Haptophyte algae of the genus *Pavlova* contain 4, 24-dimethyl-5α-cholest-22E-en-3β-ol, 4, 24-dimethyl-5α-cholestan-3β-ol, 4-methyl-24-ethyl-5α-cholest-22*E*-en-3β-ol and 4-methyl-24-ethyl-5α-cholestan-3β-ol [4]. Bacteria have ever been proposed as one of the sources of 4-methyl sterols in sediments [5], but only a few bacteria contain 4-methyl sterols [6, 7]. Some higher plants also contain C_{30} 4-methyl sterols. However, they occur generally in small amounts since the sterols are biosynthetic intermediates to other sterols [8]. These observations thus lead to the conclusion that dinoflagellates are major sources of 4-methylcholestan-3-ol and 4-tetramethylcholestan-3-ol. 4-Methylcholestan-3-ol and 4-tetramethylcholestan-3-ol may be useful biomarkers of autotrophic and heterotrophic dinoflagellates, respectively.

Resting cyst of *P. inaequale* contains 4-methylcholestan-3β-ol, 4, 24-dimethyl-5α-cholestan-3β-ol, dinosterol, dinostanol, and unknown C_{31} sterol as major free sterols (Fig. 2). Although sterol distribution of resting cyst is nearly similar to that of motile cell, the unknown C_{31} sterol occurred only in resting cyst (Fig. 2). None of motile cells contains this unknown C_{31} sterol. 4α-Methylgorgostanol is known as typical C_{31} sterol produced by dinoflagellate [8]. 4α-Methylgorgostanol was originally detected in zooxanthellae of Briareum asbestinum and dinoflagellate, *Kryptoperidinium* (= *Glenodinium*) *foliaceum* [9, 10]. However, mass spectral study shows that the unknown C_{31} sterol in the present study is clearly different from 4α-methylgorgostanol. The unknown C_{31} sterol has not been detected in the microalgal culture samples. This compound could have been produced during resting stage. Further study on this unknown C_{31} sterol will provide clues for biomarkers of dinoflagellate resting cysts.

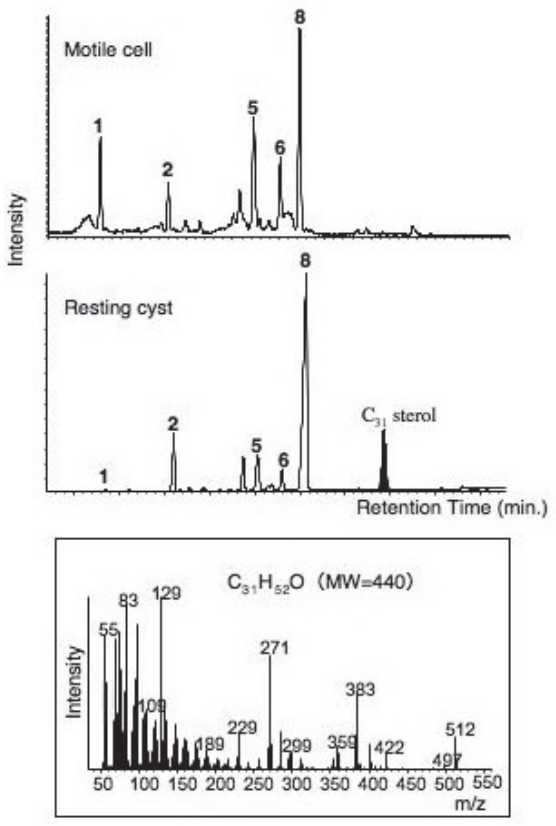

Fig. 2 Total ion chromatograms of sterol fractions from motile cell and resting cyst of *P. inaequale* and mass spectra of C_{31} sterol. 1: Cholesterol, 2: 4*α*-Methylcholestan-3*β*-ol, 5: 4*α*, 24-Dimethyl-5*α*-cholestan-3*β*-ol, 6: Dinosterol, 8: 4*α*, 23R, 24R-Trimethyl-5*α*-cholestan-3*β*-ol, 9: 4*α*-Tetramethycholestan-3*β*-ol.

CONCLUSIONS

1. The dinosterol concentration of heterotrophic dinoflagellate is about 4–12 times higher than those of autotrophic species, suggesting that the heterotrophic dinoflagellate can be an important source of dinosterol in some sediments.

2. 4-Methylcholestan-3-ol was detected only in autotrophic dinoflagellates, whereas 4-tetramethylcholestan-3-ol was confined to the heterotrophic dinoflagellates. These compounds may serve as potential biomarkers in the respective types of dinoflagellates.

3. The unknown C_{31} sterol detected in resting cyst of *P. inaequale* could be a potential biomarker of dinoflagellate resting cysts.

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