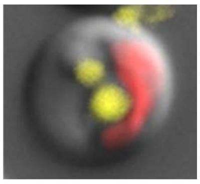
Alkenone Body:
A Novel Lipid Body Identified as a Major Carbon Storage Organelle in Alkenone-Producing Haptophyte Algae

Alkenones are known as long-chain ketones (usually C_{37-39}) with one *keto*-group, 2-4 *trans*-type double bonds in a molecule. At present, five species of haptophyte algae are known as **alkenone-producing microalgae**, such as *Emiliania huxleyi* (Figs.) and *Tisochrysis lutea*.



Alkenone-producing haptophyte algae are known to be one of sources of crude oils, natural gasses and black shales which had been produced in geological era such as the Cretaceous era. In addition, the haptophyte *E. huxleyi* classified in coccolithophores is also well-known as an organism of which calcium carbonate cell-covering (named as coccoliths) had become "limestone bed" such as **the White Cliff in Dover**, UK. We can frequently observe **a huge bloom of *E. huxleyi*** from satellite even at present at many oceanic areas, indicating *E. huxleyi* still function as one of great **CO_2-sequestrators** which transport atmospheric CO_2 to ocean sediment as the biological CO_2 pump.

Shiraiwa's group found that the haptophyte alga *E. huxleyi* alkenones and low molecular compounds (including mannitol), but not β-glucan produced generally as neutral polysaccharide carbon storage in most algae, function in **major carbon/energy storage** *in E. huxleyi*, irrespective of the growth phase and in compared with other algae the low carbon flux into β-glucan is a unique feature of carbon metabolism in *E. huxleyi* **[Article 1]**.

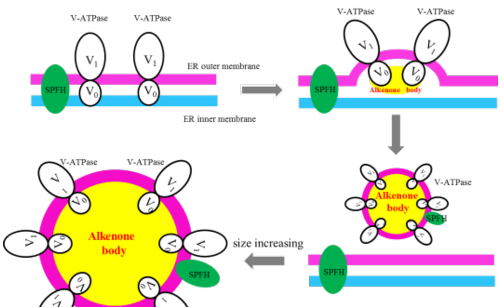
Lipid Body of *T. lutea* = Alkenone Body (AB)

Percentage (%) of total neutral lipids			
Sample no.	Alkenones (%)	Alkenes (%)	Others[1] (%)
Average	74.2±3.35	1.2±0.2	24.6±3.26

[1] Others dominantly includes sterols.

Shiraiwa's group also found that the lipid body in haptophyte alga *Tisochrysis lutea* can be more appropriately named as "**Alkenone Body (AB)**" since alkenones are predominated in the lipid body according to GC-MS analysis. Our data suggest that AB of *T. lutera* is surrounded by a lipid membrane originating from either the ER or the ER-derived four layer-envelopes chloroplast and function as the storage site of alkenones, alkenes and sterols **[Article 2]**.

Model of Alkenone Body (AB) formation

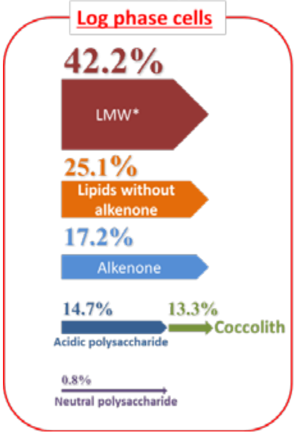


Lipid bodies in *T. lutea* are probably related to Endoplasmic reticulum (ER).

Yamane et al. (incl. Shiraiwa's group) (2013) showed that the haptophyte *E. huxleyi* generated a high yield of ***n*-alkanes** of various lengths (*n*-tridecane to*n*-pentatriacontane). The GC-MS profiles of these *n*-alkanes were similar to those of **native petroleum crude oils** despite containing a considerable amount of *n*-hentriacontane. The ratio of phytane to *n*-octadecane was also similar to that of native crude oils **[Article 3]**. This paper confirmed results of our two papers which reported the production of liquid and gaseous hydrocarbons by **pyrolysis** of the haptophyte *E. huxleyi* [**Article 4 & 5**].

	Microalgae			
	Arthrospira platensis (*Cyanobacteria*)	*Dunaliella tertiolecta* (*Chlorophyta*)	*Emiliania huxleyi* (*Haptophyta*)	*Euglena gracilis* (*Euglenophyta*)
Lipid compositions (%)[a]				
Isoprenoids	4.3	4.5	2.2	3.0
Fatty acid methyl esters	95.7	95.5	42.6	70.3
Fatty alcohols	ND	ND	ND	20.5
Wax esters	ND	ND	ND	6.2
Alkenes	ND	ND	15.0	ND
Alkenones	ND	ND	40.2	ND
Crude oil production[b] (%) in chloroform fraction	17.5 14.6*	13.2 8.7*	29.4 23.2*	20.4 17.2*
Oil recovery (%)[c] in hexane subfractions	4.2 1.2*	2.8 1.5*	8.9 5.7*	5.8 3.1*

Photosynthetic ^{14}C-labelling in *E. huxleyi*



[Article 1]

Quantitative Analysis of Carbon Flow into Photosynthetic Products Functioning as Carbon Storage in the Marine Coccolithophore, *Emiliania huxleyi*

Tsuji Y, Yamazaki M, Suzuki I, Shiraiwa Y

Marine Biotechnology 17:428–440 (2015). [Open Access]

The bloom-forming coccolithophore *Emiliania huxleyi* (Haptophyta) is a dominant marine phytoplankton, cells of which are covered with calcareous plates (coccoliths). *E. huxleyi* produces unique lipids of C_{37}–C_{40} long-chain ketones (alkenones) with two to four trans-unsaturated bonds, β-glucan (but not α-glucan) and acid polysaccharide (AP) associated with the morphogenesis of $CaCO_3$ crystals in coccoliths. Despite such unique features, there is no detailed information on the patterns of carbon allocation into these compounds. Therefore, we performed quantitative estimation of carbon flow into various macromolecular products by conducting ^{14}C-radiotracer experiments using $NaH^{14}CO_3$ as a substrate. Photosynthetic ^{14}C incorporation into low molecular-mass compounds (LMC), extracellular AP, alkenones, and total lipids except alkenones was estimated to be 35, 13, 17, and 25 % of total ^{14}C fixation in logarithmic growth phase cells and 33, 19, 18, and 18 % in stationary growth phase cells, respectively. However, less than 1 % of ^{14}C was incorporated into β-glucan in both cells. ^{14}C-mannitol occupied ca. 5 % of total fixed ^{14}C as the most dominant LMC product. Levels of all ^{14}C compounds decreased in the dark. Therefore, alkenones and LMC (including mannitol), but not β-glucan, function in carbon/energy storage in *E. huxleyi*, irrespective of the growth phase. Compared with other algae, the low carbon flux into β-glucan is a unique feature of carbon metabolism in *E. huxelyi*.

Keywords: Alkenones; Carbon partitioning; Carbon storage compound; *Emiliania huxleyi*; Haptophyte; Lipid biosynthesis.

[Article 2]

Proteomic analysis of lipid body from the alkenone-producing marine haptophyte alga, *Tisochrysis lutea*

Shi Q, Araie H, Bakku RK, Fukao Y, Rakwal R,Suzuki I and Shiraiwa Y

Proteomics, *in press. Article first published online: 2 JUL 2015 DOI: 10.1002/pmic.201500010* [Open Access]

Lipid body (LB) is recognized as the cellular carbon and energy storage organelle in many organisms. LBs have been observed in the marine haptophyte alga *Tisochrysis lutea* that produces special lipids such as long-chain (C_{37}-C_{40}) ketones (alkenones) with 2–4 *trans*-type double bonds. In this study, we succeeded in developing a modified method to isolate LB from *T. lutea*. Purity of isolated LBs was confirmed by the absence of chlorophyll auto-fluorescence and no contamination of the most abundant cellular protein ribulose-1,5-bisphosphate carboxylase/oxygenase. As alkenones predominated in the LB by GC-MS analysis, the LB can be more appropriately named as "**Alkenone Body (AB)**." Extracted AB-containing proteins were analyzed by the combination of 1DE (SDS-PAGE) and MS/MS for confident protein identification and annotated using BLAST tools at National Center for Biotechnology Information. Totally 514 proteins were identified at the maximum. The homology search identified three major proteins, V-ATPase, a hypothetical protein EMIHUDRAFT_465517 found

in other alkenone-producing haptophytes, and a lipid raft-associated SPFH domain-containing protein. Our data suggest that AB of *T. lutera* is surrounded by a lipid membrane originating from either the ER or the ER-derived four layer-envelopes chloroplast and function as the storage site of alkenones and alkenes.

Keywords: Alkenone body; Haptophyte; Lipid body; Proteome; *Tisochrysis lutea*

[Article 3]

Anaerobic coculture of microalgae with *Therosipho globiformans* and *Methanocadococcus jannaschii* at 68°C enhances generation of n-alkene-rich biofuels after pyrolysis.

Yamane, K., S. Matsuyama, K. Igarashi, M. Utsumi, Y. Shiraiwa and T. Kuwabara*

Applied and Environmental Microbiology 79 (3): 924-930 (2013). doi: 10.1128/AEM.01685-12

We tested different alga-bacterium-archaeon consortia to investigate the production of oil-like mixtures, expecting that *n*-alkane-rich biofuels might be synthesized after pyrolysis. *Thermosipho globiformans* and *Methanocaldococcus jannaschii* were cocultured at 68°C with microalgae for 9 days under two anaerobic conditions, followed by pyrolysis at 300°C for 4 days. *Arthrospira platensis*(*Cyanobacteria*), *Dunaliella tertiolecta* (*Chlorophyta*), *Emiliania huxleyi* (*Haptophyta*), and *Euglena gracilis* (*Euglenophyta*) served as microalgal raw materials. *D. tertiolecta*, *E. huxleyi*, and *E. gracilis* cocultured with the bacterium and archaeon inhibited their growth and CH_4 production. *E. huxleyi* had the strongest inhibitory effect. Biofuel generation was enhanced by reducing impurities containing alkanenitriles during pyrolysis. The composition and amounts of *n*-alkanes produced by pyrolysis were closely related to the lipid contents and composition of the microalgae. Pyrolysis of *A. platensis* and *D. tertiolecta* containing mainly phospholipids and glycolipids generated short-carbon-chain *n*-alkanes (*n*-tridecane to *n*-nonadecane) and considerable amounts of isoprenoids. *E. gracilis* also produced mainly short *n*-alkanes. In contrast, *E. huxleyi* containing long-chain (31 and 33 carbon atoms) alkenes and very long-chain (37 to 39 carbon atoms) alkenones, in addition to phospholipids and glycolipids, generated a high yield of *n*-alkanes of various lengths (*n*-tridecane to*n*-pentatriacontane). The gas chromatography-mass spectrometry (GC-MS) profiles of these *n*-alkanes were similar to those of native petroleum crude oils despite containing a considerable amount of *n*-hentriacontane. The ratio of phytane to *n*-octadecane was also similar to that of native crude oils.

[Article 4]

Liquid-saturated hydrocarbons resulting from pyrolysis of the marine coccolithophores *Emiliania huxleyi* and *Gephyrocapsa oceanica*.

Wu, Q., Y. Shiraiwa*, H. Takeda, G. Sheng and J. Fu*

Marine Biotechnology 1: 346-352 (1999).

Two nanoplanktonic marine coccolithophores, *Emiliania huxleyi* and *Gephyrocapsa oceanica*, were grown at 23°C with a 16-hour light and 8-hour darkness regimen. The cells were dried at room temperature and then subjected to pyrolysis at 100° to 500°C under anoxygenic conditions to produce hydrocarbons. Temperature-dependent profiles of the liquid-saturated hydrocarbons (saturates) produced during pyrolysis were very similar for the two strains, although the total amount was higher in *E. huxleyi* than in *G. oceanica*. The amount of saturates produced was only

0.05% to 0.15% below 200°C, but about 2.1% to 2.8% at 300°C. Their major components were normal alkanes in a series ranging from nC11 to nC35 with the predominant peak at nC_{15}. At 400° and 500°C most of saturates transformed into gaseous compounds. The major saturates identified in all pyrolysates were normal C_{31} monounsaturated and diunsaturated alkenes, a series of normal alkanes, phytenes, C_{28} sterenes, and steranes. Profiles of saturates in gas chromatography–mass spectroscopy varied with increasing pyrolysis temperature and also differed between *E. huxleyi* and *G. oceanica*. The two coccolithophores are useful candidates for the production of renewable liquid fuel through pyrolysis—especially *E. huxleyi*, which has higher production. The results also provide information for further studies on the characterization, source, and paleogeographic distribution of marine sediment.

Key words: coccolithophores, *Emiliana huxleyi, Gephyrocapsa oceanica*, pyrolysis, liquid-saturated hydrocarbons, marine sediment

[Article 5]

A renewable energy source-hydrocarbon gasses resulting from pyrolysis of the marine nanoplanktonic alga Emiliania huxleyi.

Wu, Q., Dai, J., Y. Shiraiwa, Sheng, G. and J. Fu

Journal of Applied Phycology 11: 137-142 (1999).

The marine coccolithophore, *Emiliania huxleyi*, grown in the laboratory was subjected to vacuum pyrolysis at various temperatures from 100 to 500 °C. The highest yield of pyrolytic gases (183 mL g−1 dry cells) was obtained at 400 °C. The amount of total hydrocarbon gas produced at 400 °C was 129 mL, about 10 times higher than at 300 °C. CH4 was the major component at the high gas-production stage (400–500 °C). The great increase in hydrocarbon gases at 400 °C was accompanied by a marked decrease in liquid saturates and aromatics. The results indicate that the liquid hydrocarbons (oil) produced by pyrolysis at lower temperature is a direct source for the formation of the hydrocarbon gases. Due to its large potential for the production of biomass and hydrocarbons with low energy input, *E. huxleyi* is suggested as one of candidates for the production of renewable fuels.

Key words: *Emiliania huxleyi*, hydrocarbon gases, renewable energy, pyrolysis, temperature effect