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PHYLOGENETIC SIGNIFICANCE OF THE LIMITED DISTRIBUTION OF OCTADECAPENTAENOIC ACID IN PRYMNESIOPHYTES AND PHOTOSYNTHETIC DINOFLAGELLATES

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Abstract: Fatty acid composition of lipids from two strains of prymnesiophytes, seven strains of photosynthetic dinoflagellates, one strain of non-photosynthetic dinoflagellates and two strains of cryptomonads was examined with special emphasis on the presence or the absence of an octadecapentaenoic acid [18:5(*n*–3)].

Prymnesiophytes (*Emiliania huxleyi* and *Gephyrocapsa oceanica*) and photosynthetic dinoflagellates [*Amphidinium carterae*, *Prorocentrum micans*, *Protoceratium reticulatum*, *Pyrocystis lunula*, *Scrippsiella trochoidea*, *Symbiodinium microadriaticum* (two strains)] examined all contained significant levels of 18:5(*n*–3) as a fatty acyl component. However, a non-photosynthetic dinoflagellate (*Gymnodinium* sp.) and cryptomonads (*Cryptomonas* sp. and *Rhodomonas* sp.) did not contain any level of 18:5(*n*–3). These results suggest that 18:5(*n*–3) is highly reliable as a specific marker of prymnesiophytes and photosynthetic dinoflagellates.

18:5(*n*–3) was found in glycolipids (monogalactosyl-diacylglycerol and digalactosyldiacylglycerol) and in phospholipids (phosphatidyl-choline) both in prymnesiophytes and dinoflagellates. Levels of 18:5(*n*–3) in glycolipids were higher than those in phospholipids, particularly in dinoflagellates.

1. Introduction

All-*cis*-Δ-3,6,9,12,15-octadecapentaenoic acid [18:5(*n*–3)], an unsaturated fatty acid, has been found only in prymnesiophytes (VOLKMAN *et al.*, 1981; NAPOLITANO *et al.*, 1988) and photosynthetic dinoflagellates (JOSEPH, 1975; HARVEY *et al.*, 1988). In a previous study (OKUYAMA *et al.*, 1992b) we identified one of major unsaturated fatty acids of prymnesiophytes as 18:5(*n*–3) and suggested that 18:5(*n*–3) is a specific marker of prymnesiophytes, implying that chloroplasts of photosynthetic dinoflagellates containing 18:5(*n*–3) might have come from prymnesiophytes. In order to confirm the possibility, examination of the distribution of 18:5(*n*–3) in much more algal samples as well as the morphology of organelles and genomic resemblance in prymnesiophytes and dinoflagellates has been required. There are several lines of circumstantial evidence (TOMAS and COX, 1973; JEFFREY and VESK, 1976; TANGEN and BJØRNLAND, 1981; WILCOX and WEDEMAYER, 1984; WATANABE *et al.*, 1987) that photosynthetic dinoflagellates harbor various microalgae (chrysophyte, prymnesiophyte, cryptomonad and chlorophylls *a* and *b*-containing alga) as endosymbionts. However, only some species of dinoflagellates which have the particular morphology and pigments have

been examined on relationships between the endosymbionts and other algal groups. In this study further examinations were carried out to show the phylogenetic significance of 18:5(*n*–3) as a specific and common marker of prymnesiophytes and photosynthetic dinoflagellates.

2. Materials and Methods

2.1. Algal strains

Prymnesiophytes: *Emiliania huxleyi* was isolated from waters of the northwest Pacific Ocean (44°N, 155°E) and *Gephyrocapsa oceanica* (strain no. NIES-353) was purchased from the NIES-Collection (National Institute for Environmental Studies, Tsukuba, Ibaraki Prefecture). *Isochrysis* sp. and *Prymnesium* sp. were given by I. INOUYE of Tsukuba University and H. KAWAI of Hokkaido University, respectively. Prymnesiophyte strain B, a cold stenothermic marine alga was originally isolated from seawaters off Antarctica (OKUYAMA *et al.*, 1992a). Photosynthetic dinoflagellates: *Amphidinium carterae* and *Symbiodinium microadriaticum* (D-3), identified by T. HORIGUCHI of Sinshu University, were isolated from seawaters off Sesokojima island, Okinawa Prefecture and from seawaters off Sadogashima island, Niigata Prefecture, respectively. *Pyrocystis lunula* and *Symbiodinium microadriaticum* (Y-6) were given by E. NAKAMURA of Hokkaido University. *Protoceratium reticulatum* (strain no. NIES-318) and *Scrippsiella trochoidea* (strain no. NIES-369) were purchased from the NIES-Collection. Non-photosynthetic dinoflagellates: *Gymnodinium* sp., identified by T. HORIGUCHI, was isolated from seawaters of the shore of Kurahashi, Hiroshima Prefecture. Cryptomonads: *Cryptomonas* sp. was isolated from a pond in the Center of Experimental Plants and Animals of Hokkaido University and *Rhodomonas* sp. was isolated from seawaters off Kasadojima island, Yamaguchi Prefecture. Isolation of algae from samples collected was carried out by repeated pipetting.

2.2. Growth conditions

The cells were grown photoautotrophically at 5°C for prymnesiophyte strain B and at 20°C for the remaining algae except for those of *Gymnodinium* sp. and *Cryptomonas* sp. in PROVASOLI's ES medium (PROVASOLI, 1968) with a 16-h light/8-h dark regime under daylight fluorescent light at 55 μmol $m^{-2}s^{-1}$. For *Gymnodinium* sp., bialgal cultures with a strain of diatoms were carried out at 20°C under the lighting condition as described above, where *Gymnodinium* sp. was fed with living diatoms. The diatom is an unidentified strain (strain no. MIZ-38) isolated by M. MIZUNO of Tokyo University of Agriculture. *Cryptomonas* sp. was grown photoautotrophically at 20°C in tap water that contains 1% (v/v) ES enrichment (PROVASOLI, 1968).

Abbreviations: MGD, monogalactosyldiacylglycerol; DGD, digalactosyldiacylglycerol; PC, phosphatidylcholine; TLC, thin-layer chromatography; for fatty acids, the number before the colon indicates the length of the carbon chain and the number after the colon indicates the number of double bonds in the chain; *n*–number in a parenthesis represents the number of carbon atoms from the last double bond to the terminal methyl group.

2.3. Extraction and analysis of lipids

Total lipids were extracted from wet cells by the method of BLIGH and DYER (1959). The lipid classes were separated by two-dimensional TLC on precoated silica gel plates (5721; Merck, Darmstadt, FRG) with mixtures of acetone, benzene, methanol and water (8:3:2:1, by volume), and chloroform, methanol and 25% NH_4 (OH) in water (13:7:1, by volume) for the first and second dimensions, respectively (SATO, 1991). After fluorometric detection with primuline under ultraviolet light, the areas of silica gel of the plates corresponding to individual classes of lipids were scraped off. Identification of lipid classes was based on the results of cochromatography with authentic standards during TLC and the reactions of each lipid on TLC plates with stains specific for sugars, phosphates, amino groups and choline (OKUYAMA *et al.*, 1992b). The total lipids and individual lipids with silica gel were methanolized with 10% (v/v) acetyl chloride in methanol in the presence of octadecanoic acid as an internal standard for 1 h at 85°C. The resultant fatty acid methyl esters were analyzed by a gas-liquid chromatography as described previously (OKUYAMA *et al.*, 1992b). The separated fatty acid methyl esters were identified by gas chromatography–mass spectrometry as described previously (OKUYAMA *et al.*, 1992b).

2.4. Chemicals

Authentic standards of glycerolipids (PC, MGD, DGD) and fatty acids [14:0, 16:0, 18:2(*n*–6), 18:3(*n*–3), 18:3(*n*–6) and 18:4(*n*–3)] are products of Sigma Chemical Co. (St. Louis, MO, USA). 18:5(*n*–3) was isolated and purified from cells of prymnesiophyte strain B as described by OKUYAMA *et al.* (1992b).

3. Results and Discussion

As shown in Table 1, both strains of prymnesiophytes, *Gephyrocapsa oceanica* (strain no. NIES-353) and *Emiliania huxleyi*, contained 11% and 12% of 18:5(*n*–3) in the total fatty acids, respectively. These results agreed with the results examined by OKUYAMA *et al.* (1992b) for *G. oceanica* and by VOLKMAN *et al.* (1981) for *E. huxleyi*. Although three strains of prymnesiophytes, *Isochrysis* sp., *Prymnesium* sp. and prymnesiophyte strain B in Table 1, were previously confirmed to have 18:5(*n*–3) by us (OKUYAMA *et al.*, 1992b), in the present study these strains were also examined on levels of 18:5(*n*–3) in lipid classes (Table 2). Seven strains of photosynthetic dinoflagellates all contained 18:5(*n*–3), but, its level significantly varied according to species (Table 1). In *Symbiodinium microadriaticum* (D-3), *S. microadriaticum* (Y-6) and *Scrippsiella trochoidea*, 18:5(*n*–3) accounted for 15%, 18% and 20% of respective total fatty acids. Whereas in *Amphidinium carterae*, *Pyrocystis lunula* and *Protoceratium reticulatum*, its levels were less than 5%. In contrast, the non-photosynthetic dinoflagellate (*Gymnodinium* sp.) which was fed with living diatoms did not have any level of 18:5(*n*–3) (Table 1). According to HENDERSON *et al.* (1988), a marine dinoflagellate, *Crypthecodinium cohnii* grown heterotrophically contained no 18:5(*n*–3), which was the first example showing that 18:5(*n*–3) is not found in non-photosynthetic dinoflagellates. Our strain of non-photosynthetic dinoflagellate is the second example in this respect.

Table 1. Levels of 18:5(n–3) in total lipids in prymnesiophytes and dinoflagellates.

Organism	% of 18:5(n–3) in total lipids
Prymnesiophytes	
Emiliania huxleyi	11
Gephyrocapsa oceanica	12
Isochrysis sp.	6
Prymnesium sp.	15
Prymnesiophyte strain B	22
Dinoflagellates (photosynthetic)	
Amphidinium carterae	3
Prorocentrum micans	34
Protoceratium reticulatum	5
Pyrocystis lunula	3
Scrippsiella trochoidea	20
Symbiodinium microadriaticum (D-3)	15
Symbiodinium microadriaticum (Y-6)	18
Dinoflagellates (non-photosynthetic)	
Gymnodinium sp.	0
Cryptomonads	
Cryptomonas sp.	0
Rhodomonas sp.	0

We previously suggested (OKUYAMA *et al.*, 1992b) that chloroplasts of dinoflagellates might have come from prymnesiophytes, since 18:5(*n*–3) is found only in prymnesiophytes and photosynthetic dinoflagellates. The fact that 18:5(*n*–3) was not found in non-photosynthetic dinoflagellates supports the hypothesis, with which our new results are not in conflict. It is possible that chloroplasts of most photosynthetic dinoflagellates are derived from prymnesiophytes, since all of them examined by us have 18:5(*n*–3). Two strains of cryptomonads, *Rhodomonas* sp. and *Cryptomonas* sp., did not contain any level of 18:5(*n*–3) (Table 1). Cryptomonads are also regarded as microalgae that harbor endosymbionts (DOUGLAS *et al.*, 1991). Since the presence of 18:5(*n*–3) has not been reported in any species of cryptomonads including two strains in the present study, it is unlikely that cryptomonads capture prymnesiophytes as endosymbionts.

Total lipids from three strains of prymnesiophytes and four strains of photosynthetic dinoflagellates were separated into individual lipid classes by two-dimensional TLC. In all the organisms examined, MDG, DGD and PC were dominant polar lipids (Table 2). 18:5(*n*–3) was found in glycolipids (MGD and DGD) from all species of prymnesiophytes and photosynthetic dinoflagellates examined in the present study. In prymnesiophytes 18:5(*n*–3) was contained also in PC, although its level was very low (less than 0.5%) in PC of *Isochrysis* sp. which contained low level of 18:5(*n*–3) in total lipids. In photosynthetic dinoflagellates, *Scrippsiella trochoidea* and *Prorocentrum micans* contained PC to which 18:5(*n*–3) were bound, whereas PC from *Amphidinium carterae* and *Symbiodinium microadriaticum* contained no 18:5(*n*–3) or, if any, its level was negligible (Table 2). In *A. carterae* level of 18:5(*n*–3) in total lipids was also very low (Table 1). Levels of 18:5(*n*–3) in PC were generally lower than those in MGD and DGD especially in photosynthetic dinoflagellates.

Table 2. Levels of MDG, DGD and PC in prymnesiophytes and dinoflagellates and levels of 18:5(n–3) in these lipid classes.

Organism	Level of lipid class [Level of 18:5(*n*–3) in each lipid class]		
	MGD	DGD	PC
Prymnesiophytes			
Isochrysis sp.	35%	9%	15%
	[17%]	[9%]	[Tr*]
Prymnesium sp.	34%	9%	10%
	[24%]	[10%]	[18%]
Prymnesiophyte strain B	21%	19%	10%
	[26%]	[32%]	[23%]
Dinoflagellates			
Amphidinium carterae	11%	26%	14%
	[14%]	[1%]	[0%]
Prorocentrum micans	21%	22%	29%
	[82%]	[40%]	[7%]
Scrippsiella trochoidea	10%	12%	15%
	[61%]	[19%]	[9%]
Symbiodinium microadriaticum (D-3)	19%	25%	17%
	[51%]	[14%]	[Tr]

*Tr, Less than 0.5%.

Glycolipids are abundant components of chloroplasts, in particular of thylakoid membranes, and rare in membranes other than membranes of chloroplasts (HARWOOD and RUSSELL, 1984). Thus, it is suggested from the high abundance of 18:5(*n*–3) in glycolipids of prymnesiophytes and photosynthetic dinoflagellates that 18:5(*n*–3) is contained in their chloroplasts as a fatty acyl component of thylakoid and/or envelope membrane. This implies that chloroplasts of prymnesiophytes reside in the cell of photosynthetic dinoflagellates. In general, PC is located in various membranes such as envelopes of chloroplasts, membranes of mitochondria, endoplasmic reticulum and cytoplasmic membrane (HARWOOD and RUSSELL, 1984). However, it appears that thylakoid membranes contain no PC (DORNE *et al.*, 1990). At the present time, the localization of 18:5(*n*–3)-containing PC in prymnesiophytes is still not defined. However, if chloroplasts of photosynthetic dinoflagellates could be originated from prymnesiophytes, it is likely that 18:5(*n*–3)-containing PC in photosynthetic dinoflagellates might be present only in their chloroplast envelopes.

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