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DIACYLGLYCERYLTRIMETHYLHOMOSERINES AND PHOSPHOLIPIDS OF SOME GREEN MARINE MACROPHYTES

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Key Word Index—Green marine macrophytes; phospholipids; diacylglyceryltrimethylhomoserines.

Abstract—The composition of the phospholipids and the distribution of diacylglyceryltrimethylhomoserines (DGTS) has been investigated in 15 species of green marine seaweeds. The total lipid content of the seaweeds varied from 0.8 to 4.3 mg/g dry weight, phospholipids constituting from 3.8 to 27.8% of the total. The content of DGTS varied from 4 to 111 μg/g dry weight.

INTRODUCTION

Various species of seaweed add much to the quality of the traditional food of people in many countries and therefore they have become the subject of much attention recently [1]. Phospholipids of seaweeds are poorly investigated and are the subject of only few studies [2–8]. However, Pohl and Zurheide [9] and Khotimchenko and Svetashev [10] have conducted a detailed survey of marine algal lipids and fatty acids. 1(3),2-Diacylglyceryl-(3)-*O*-4′-(*N*,*N*,*N*-trimethyl)homoserines (DGTS) have been repeated in some microalgae [2–8, 11–14], macrophytes [15], phytoflagellates [16], a human pathogenic fungus [17] and Protozoa [18] and ferns [19]. Sato and Furuya [20, 21] have also has described the distribution of these compounds in selected species of vascular and green plants.

The present investigations of phospholipids and DGTS in selected marine macrophytes was conducted to widen our knowledge about such compounds from this source.

RESULTS AND DISCUSSION

Phospholipid composition of 13 species of seaweeds belonging to the order Chlorophyta and two species of seagrasses belonging to the order Embryophyta were determined. The results showed that green seaweeds can be divided in two groups according to their phospholipid composition. The first group includes representatives of the class Ulotrichophyceae. The main feature of these seaweeds is that they do not contain PC. The macrophytes of this group contain PE, PI, PS, PA and a large amount of DGTS. One of the main phospholipids in seaweeds included in the second group belonging to the class Siphonophyceae is PC, its content varying from 17.9 to 25.3% of the total phospholipids. This group also contains a large quantity of PA. Both groups are characterized by a high content of PG, the amount of this phospholipid varying from 22.1 to 47%. DGTS is a distinguishing feature with all of the investigated seaweed except for the seagrasses. It should be noted that seaweeds which lack PC have three to five times more DGTS than those which contain PC.

Seagrasses belonging to the genus Zosteraceae do not differ much from higher plants in terms of their phospholipid composition; they show a high content of PC (43–45%), low levels of PG (13–15%), a high content of phospholipids (> 20%) and the absence of DGTS (Table 1).

The choice of chromatographic systems used to separate glyco- and phospholipids is important in investigations of the lipid composition of marine macrophytes. These lipids classes are well resolved using the chromatographic system of Pohl *et al.* [22]. However, when working with seaweed lipids the use of neutral or alkaline mixtures of solvents does not yield good results because sulphoquinovosyl diacylglycerols are lost. That is why we have chosen the acidic system of solvents proposed by Vaskovsky and Khotimchenko [23], viz., first direction, chloroform–acetone–methane–formic acid–water (150:20:10:10:4), second direction, acetone–benzene–formic acid–water (200:30:3:10). Spot compactness and resolution was observed to increase with the use of plates coated with silica gel and Florisil. To identify PA other solvent systems were used viz., first direction, chloroform–methanol–benzene–28% ammonia (65:30:10:6) second direction, chloroform–methanol–benzene–acetone–acetic acid–water (170:30:10:5:4:1). These systems are good for the separation of the main phospholipids and identification of PA although resolution between digalactosyl diacylglycerols and sulphoquinovosyl diacylglycerols does not occur.

Thus, our investigations have shown that PG is the major phospholipid of marine macrophyte polar lipids. Practically all green seaweeds also contain DGTS and PS. Seagrasses differ from seaweeds in terms of their phospholipid composition. Representatives of the class Ulotrichophyceae do not contain PC.

EXPERIMENTAL

All the marine macrophytes were collected in July 1987 in the north-east part of the Black Sea (Feodosiya and Karadag Bays).

Seaweeds and seagrasses were heated for 2–3 min in boiling H_2O and lipids extracted by a modification of the method of ref.

Table 1. Phospholipid and diacylglyceryltrimethylhomoserine composition of some green marine macrophytes

Plant material	TL*	PL†	PG	PE	PC	PI	PS	PA	DPG	DGTS‡
Order Chlorophyta										
Class Ulotrichophyceae										
Enteromorpha prolifera (Müll.) J. Ag.	0.8	8.4	28.0	10.4	—	12.2	9.7	4.0	—	36.0 (24.2)§
Enteromorpha linza (L.) J. Ag.	1.2	6.9	26.0	11.2	—	10.2	8.9	3.8	—	39.3 (32.5)
Urospora penicilliformis (Roth.) Aresch.	2.3	6.9	25.4	11.9	—	7.2	9.3	4.2	—	42.0 (66.7)
Ulotrix flacca (Dillw.) Thur.	1.6	4.8	22.1	14.3	—	9.6	13.6	2.2	—	38.2 (29.3)
Entocladia viridis Reinke	1.8	7.3	23.5	12.9	—	10.3	10.1	1.3	—	41.9 (55.1)
Ulva rigida (L.) Ag.	2.6	8.4	25.0	10.3	—	5.4	8.3	—	—	51.0 (111.4)
Ulva sp. (L.)	2.1	9.1	21.3	9.6	—	4.9	7.7	—	—	56.5 (108.0)
Class Siphonophyceae										
Cladophora vagabunda (Kütz).	2.2	18.4	47.0	7.5	23.8	8.0	6.5	2.0	—	5.2 (20.7)
Cladophora sericea (Huds.) Kütz.	2.0	16.7	46.8	8.8	21.3	6.6	7.9	—	2.1	6.5 (21.7)
Chaetomorpha crassa (Ag.) Kütz.	1.8	3.8	32.1	6.9	17.9	17.2	6.4	7.2	6.0	6.3 (4.3)
Bryopsis plumosa (Huds.) Ag.	1.9	14.3	32.2	12.3	24.8	11.3	5.2	—	—	14.2 (38.6)
Bryopsis hypnoidea Lamour.	2.8	19.1	35.5	9.4	23.7	12.8	4.0	—	—	11.8 (63.1)
Rhizoclonium implexum (Dillw.) Kütz.	2.9	14.5	41.6	5.9	25.3	7.0	10.0	—	3.6	6.6 (27.8)
Order Embryophyta										
Genus Zosteraceae										
Zostera marina L.	4.3	24.3	13.9	23.9	43.1	12.2	6.9	—	—	—
Zostera nana Roth.	3.9	27.8	15.5	24.3	45.1	10.3	4.8	—	—	—

*TL Total lipids, mg/g dry wt.

†PL Content of phospholipids as a proportion of total lipids.

‡DGTS Diacylglyceryltrimethylhomoserine (amount determined from N content, P content calculated on PE basis. Sum of PE and DGTS taken as 100% when determining N content).

§Content of DGTS, μg/g dry wt.

Abbreviations: PG–phosphatidylglycerol, PE–phosphatidylethanolamine, PC–phosphatidylcholine, PI–phosphatidylinositol, PS–phosphatidylserine, PA–phosphatidic acid, DPG–cardiolipin.

[24]. Ground material was extracted using published procedures and the residue re-extracted ×2–5 with small portions of $CHCl_3$–MeOH (1:1) [25]. Phospholipids were isolated by micro-TLC and P determined in them by the method of ref. [26]. N in DGTS and PE was determined according to method of ref. [27]. When carrying out qualitative determination of phospholipids as well DGTS and PE for N, 4–6 parallel determinations were made; mean values for each lipid class are given in Table 1.

To identify seaweed lipids authentic standards of glyco- and phospholipids were used. MGDG, DGDG, SQDG and DGTS were sepd from *Nephrochloris salina* cultivated in our laboratory. DGTS was identified by IR, ^1H NMR and MS as described earlier [28]. To identify phospholipids and glycolipids on chromatographic plates the following specific spray reagents were used: for free amino groups, a 0.2% soln of ninhydrin in Me_2CO; for choline-containing lipids—Dragendorf's reagent; for P-containing lipids—modified Jungnickel's reagent [29]; for phospholipids—Vaskovsky's *et al.* reagent [26]; for glycolipids—Rhodamine 6G and anthrone reagent [30] and for PG—periodate Shiff's reagent. Glyco- and phospholipids were also identified from their R_f values. Total lipids were determined according to the procedure of ref. [31].

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