PHOSPHOLIPID DISTRIBUTION AND PHOSPHOLIPID FATTY ACIDS IN FOUR SAUDI RED SEA SPONGES

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ABSTRACT

Sponge phospholipids and phospholipid fatty acids were investigated in sponges collected in Saudi Red Sea, namely *Cinachyrella* sp_1, *Cinachyrella* sp_2, *Chalinula saudiensis* and *Stylissa carteri*. More than fifty fatty acids have been identified as methyl esters and *N*-acyl pyrrolidides in each fatty acid mixture by GC/MS. The presence of bacteria was evidenced from the relatively high proportions of phosphatidylglycerol and phosphatidylinositol, and the high levels of the branched short-chain fatty acids. *Cinachyrella* sponges contained eighteen typical Δ5,9 fatty acids, and new compounds, namely 17-methyltetracosanoic, 18-methyl tetracosanoic, 18-methylpentacosanoic, 18-methylhexacosanoic, 18,24-dimethylheptacosanoic and 6-bromo-Δ5,9-nonacosadienoic acids. Phospholipid fatty acids from *Stylissa carteri* were characterised by a high content of Δ5,9 fatty acids (55.5 %) and phytanic acid (20 %). *Chalinula saudiensis* contained several Δ5,9 fatty acids, including the rare 6-bromo-Δ5,9-octacosadienoic acid. Unexpected polyunsaturated fatty acids occurred in the two latter sponges, such as arachidonic and docosahexaenoic acids.

KEY WORDS

Sponge phospholipids, Red Sea, polyunsaturated fatty acids, branched-chain fatty acids, Δ5,9-diunsaturated fatty acids, Porifera.

INTRODUCTION

Marine sponges, have proved to be a rich source of many unusual phospholipid fatty acids. These acids are quite unusual and possess long chain (23-34 carbon atoms) and unique unsaturation pattern (Δ5,9) (DJERASSI & LAM, 1991). Thus, they contrast sharply with their common counterparts where the methylene-interrupted unsaturation pattern is found in numerous other organisms. The common polyunsaturated fatty acids (n-3) et (n-6) are quite rare in sponges. Phospholipid fatty acids were reported from *Cinachyrella* sponges originating from Senegal and New-Caledonia (BARNATHAN *et al.,* 1992, 1994). As part of our ongoing investigations (BARNATHAN *et al.,* 1996; BARNATHAN & KORNPROBST, 2000), phospholipids were analysed in sponges collected by scuba diving in Saudi Red Sea: *Chalinula saudiensis*,

Stylissa carteri, *Cinachyrella* sp1 and *Cinachyrella* sp2 (RÜTZLER, 1987). *Chalinula saudiensis* is a new sponge recently described (VACELET *et al.,* 2001). The sponge species are assigned according to the Systema Porifera (HOOPER & VAN SOEST, 2002; VAN SOEST *et al.,* 2002).

MATERIAL AND METHODS

Cinachyrella sp1 (Uliczca, 1929) and *Cinachyrella* sp2 (Uliczca, 1929) (Tetillidae)**,** *Stylissa carteri* (Dendy, 1989) (Dictyonellidae) and *Chalinula saudiensis* Vacelet *et al.,* 2001 (Haplosclerida, Chalinidae) were collected by hand (Scuba) in the Red Sea off Jeddah, at depth of 10 - 20 m. Sponges were steeped in CH_2Cl_2/MeOH (1:1, v/v) and the combined extracts yielded the crude total lipids. Phospholipids were separated from other lipids by column chromatography on silica gel with hexane, dichloromethane (neutral lipids), acetone (glycolipids), methanol (phospholipids). Phospholipids were subjected to acidic methanolysis. Fatty acid methyl esters were then treated by pyrrolidine/acetic acid. High performance thin layer chromatography (HPTLC) phospholipid analysis: plates precoated with silica gel 60F 254 (Merck), Linomat IV (Camag) for standards and sample application, primulin and molybdene blue for revelation, and TLC IL scanner, automated multiple development apparatus system consisting of CH_2Cl_2/MeOH (decreasing polarity). Gas chromatography-mass spectrometry (GC-MS) was performed on a HP 5890II chromatograph, DB-1 column (30 m x 0.25 mm, 0.33 µm phase thickness) linked to a HP 5989A spectrometer and a HP 98785A integrator.

RESULTS

In sponges, phosphatidylethanolamine was usually found as major class (DJERASSI & LAM, 1991). As shown in Tab. I, phosphatidylcholine was the major class in *Cinachyrella* sponges. High levels of phosphatidylglycerol and phosphatidylinositol revealed the important presence of bacteria in these sponges. This observation was confirmed by the detection of a lot of typical bacterial short-chain and branched fatty acids such as *iso*- and *anteiso*-pentadecanoic, *iso*- and *anteiso*-heptadecanoic.

Tab. I. Phospholipid composition of *Cinachyrella* sponges

Phospholipids	*Cinachyrella* sp1	*Cinachyrella* sp2
Lyso phosphatidylcholine	2.6	--
Phosphatidylcholine	48.0	44.5
Phosphatidylethanolamine	6.7	14.9
Phosphatidylinositol	17.0	19.1
Phosphatidylglycerol	12.1	13.5
Phosphatidylserine	8.5	8.0
Diphosphatidylglycerol	5.1	- -

Fatty acid derivative mixtures were analysed by GC/MS. Mass spectra of fatty acid methyl esters and *N*-acyl pyrrolidides (ANDERSSON, 1978; WALKUP *et al.,* 1981; BARNATHAN *et al.,* 1992; CARBALLEIRA & EMILIANO, 1993) showed a major fragment ion due to Mac Lafferty rearrangement at m/z 74 and 113, respectively, as well as a molecular fragment ion, and allowed detection and location of methyl branching and unsaturation in fatty acids (*N*-acyl pyrrolidides An ion at m/z 81 (methyl esters) and m/z 180 (pyrrolidides) was characteristic for Δ5,9 unsaturation. Each fatty acid derivative was identified by its GC mobility (Equivalent chain length, ECL) and its mass spectrum.

Tab. II. Phospholipid saturated and monounsaturated fatty acids from *Cinachyrella* sp_1, **A**, and *Cinachyrella* sp_2, **B** (minor fatty acids unreported).

Fatty acids	**A**[a]	**B**[a]
octanoic (8:0)	1.3	1.4
decanoic (10:0)	1.5	2.3
tetradecanoic (14:0)	1.4	2.1
4,8,12-trimethyltridecanoic (br-16:0)	8.5	7.1
13-methyltetradecanoic (i-15:0)	0.9	6.9
12-methyltetradecanoic (a-15:0)	-	3.1
pentadecanoic (15:0)	0.4	1.3
10,13-dimethyltetradecanoic (br-16 :0)	-	1.5
14-methylpentadecanoic (i-16:0)	1.3	2.5
9-hexadecenoic (16:1)	1.8	3.4
hexadecanoic (16:0)	8.5	8.1
10-methylhexadecanoic (br-17:0)	3.9	-
13-methylhexadecanoic (br-17:0)	-	10.5
15-methylhexadecanoic (i-17:0)	1.7	3.7
14-methylhexadecanoic (a-17:0)	1.8	1.2
11-heptadecenoic (17:1)	-	1.0
heptadecanoic (17:0)	0.9	1.3
9-octadecenoic (18:1)	3.0	1.7
11-octadecenoic (18:1)	3.0	2.5
octadecanoic (18:0)	12.5	7.3
11-methyloctadecanoic (br-19:0)	2.8	0.2
17-methyloctadecanoic (i-19:0)	1.0	1.5
9-nonadecenoic (19:1)	-	1.7
11-nonadecenoic (19:1)	1.8	-
nonadecanoic 19:0	1.4	0.3
17-tetracosenoic (24:1)	0.7	0.6
17-methyltetracosanoic (br-25:0)[b]	-	4.3
18-methyltetracosanoic (br-25:0)[b]	4.5	-
18-methylpentacosanoic (br-26:0)[b]	0.6	-
19-hexacosenoic (26:1)	3.7	4.1
18-methylhexacosanoic (br-27:0)[b]	7.4	-
25-methyl-19(or 20)-hexacosenoic (i-27:1)	1.3	-
18,24-dimethylhexacosanoic (br-28:0)[b]	0.9	-
20-heptacosenoic (27:1)	3.0	-
nonacosanoic (29:0)	2.1	-

Δ5,9 fatty acids

Fatty acids	**A**	**B**
5,9-octadecadienoic (18:2)	0.4	4.2
24-methyl-5,9-pentacosadienoic (i-26:2)	-	0.4
5,9-hexacosadienoic (26:2)	0.2	0.4
25-methyl-5,9-hexacosadienoic (i-27:2)	-	0.5
24-methyl-5,9-hexacosadienoic (a-27:2)	-	2.0
5,9-heptacosadienoic (27:2)	0.6	0.5
25-methyl-5,9-heptacosadienoic (a-28:2)	-	3.0
5,9-octacosadienoic (28:2)	4.0	0.8
27-methyl-5,9-octacosadienoic (i-29:2)	0.6	-
5,9-nonacosadienoic (29:2)	1.0	tr.
6-bromo-5,9-heptacosadienoic (Br-27:2)	0.2	tr.
5,9,23-triacontatrienoic (30:3)	1.0	0.2
6-bromo-5,9-octacosadienoic (Br-28:2)	1.0	tr.
6-bromo-5,9-nonacosadienoic (Br-29:2)[b]	0.2	-

br: branched; i: *iso*; ai: *anteiso*; tr.: traces (< 0.1 %). [a]Abundance in weight. [b]Unprecedented as natural compounds.

Cinachyrella sponges

Tab. II shows almost all phospholipid fatty acids. The demospongic acids (≥ 23 C) accounted for 17.8 % in *Cinachyrella* sp_2 and 35.2 % in *Cinachyrella* sp_1. It appears from Tab. II that the phospholipid fatty acid compositions of both *Cinachyrella* are quite similar, including thirteen Δ5,9 fatty acids accounting for about 11 % in both sponges. As observed, both sponges contained a large range of *iso* and *anteiso* fatty acids typical of bacteria. Other identified branched short-chain fatty acids were the 10-methylhexadecanoic, 13-methylhexadecanoic, 10-methyloctadecanoic and 12-methyleicosanoic acids, likely originating from bacteria (VACELET, 1975; BOBBIE & WHITE, 1980; WALKUP *et al.,* 1981; GILLAN *et al.,* 1988).

In *Cinachyrella* sp_1, three new homologous 18-methyl branched acids accounted for 13.4 % of the total acids with 25 (ECL = 24.38), 26 (ECL = 25.35) and 27 (ECL = 26.38) carbon atoms, respectively, were readily identified from the mass spectra of their pyrrolidides (Fig. 1). The new 18,24-dimethylhexacosanoic acid was identified as shown in Fig. 1.

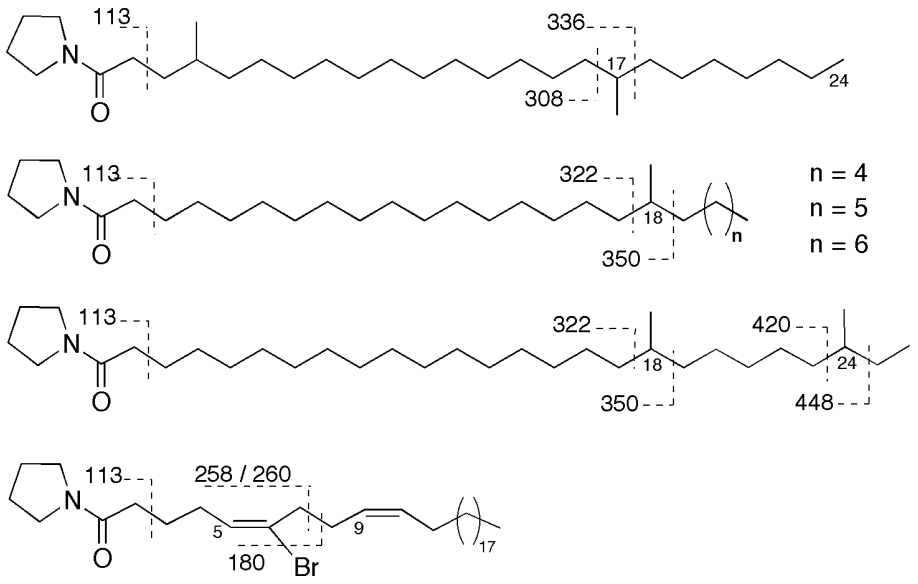

Fig. 1. Main MS fragmentation (*m/z*) of the new fatty acids (pyrrolidides).

The corresponding molecular ions were present and the corresponding fragment ion at *m/z* 336 was diminished and flanked by elevated peaks at *m/z* 322 and *m/z* 350, clearly pointed towards the methyl group at C-18. The new 17-methyltetracosanoic acid was identified in *Cinachyrella* sp_2 (4.3 %) from the mass spectrum of its pyrrolidide (ECL = 24.33) (Fig. 1) since the molecular ion was at *m/z* 435 and the corresponding fragmentation at *m/z* 322 was diminished and flanked by elevated peaks at *m/z* 308 and *m/z* 336 pointed towards the methyl group at C-17. The infrared spectrum of the fatty acid methyl esters indicated the absence of (*E*)-unsaturation. The new 6-bromo-5,9-nonacosadienoic acid was characterized as the

pyrrolidide derivative (Fig. 1) since the mass spectrum showed the usual base peak at *m/z* 113, the brominated ions at *m/z* 258 and 260 of equal intensity due to the double allylic cleavage between C-7 and C-8 positions, and an enhanced ion at *m/z* 180 due to the same cleavage after bromine loss, and the ion at *m/z* 486 after bromine loss from the molecular ion (WIJEKOON *et al.,* 1984; CARBALLEIRA & EMILIANO, 1993; BARNATHAN *et al.,* 1994).

Stylissa carteri

An unusual phospholipid fatty acid composition was observed as shown in Tab. III. Among fifty identified acids, only two of them represented 68 % of the total: 5,9-hexacosadienoic acid and phytanic acid (3,7,11,15-tetramethyl-16:0) accounting for 41 % and 20 % respectively. Polyunsaturated fatty acids quite unsual in sponges have been identified, namely Δ4,7,10,13,16-22:5, Δ9,12,15,18-22:4, Δ5,8,11,14-20:4 (arachidonic). Furthermore, we found a series of 2-hydroxylated saturated fatty acids with 23 to 26 carbon atoms.

Tab. III. Phospholipid fatty acids composition of *Stylissa carteri*

Fatty acids	ECL	%
i-16:0	15.60	1.0
9-16:1	15.77	1.3
16:0	16.00	4.1
9-17:1	16.80	0.7
17:0	17.00	0.5
3,7,11,15-TM-16:0	17.22	0.1
9,12-18:2	17.50	0.5
9-18:1	17.60	0.9
11-18:1	17.70	0.9
18:0	18.00	2.7
Δ5,8,11,14-20:4	18.93	2.5
11,15-20:2	19.70	0.8
11-20:1	19.87	0.5
Δ4,7,10,13,16-22:5	20.80	1.9
Δ9,12,15,18-22:4	20.99	0.5
17-23:1	23.06	tr
2-OH-22:0	23.17	tr
5,9-24:2	23.56	0.3
24:0	24.00	1.3
5,9-25:2	24.47	6.0
2-OH-24:0	25.27	1.8
5,9-26:2	25.52	41.5
9-26:1	25.80	8.6
2-OH-25:0	26.28	1.4
5,9-27:2	26.50	0.6
2-OH-26:0	27.28	tr
5,9-28:2	27.33	0.6

ECL = Equivalent chain length

Chalinula saudiensis

The major fatty acids were hexadecanoic, 9,12-octadecadienoic, octadecanoic, nonadecanoic, eicosanoic, docosanoic, tricosanoic and tetracosanoic acids. Several typical Δ5,9 fatty acids have been identified, including 5,9-octacosadienoic acid as major component of the series, and the unusual 6-bromo-5,9-octacosadienoic acid. These acids were identified as already explained. Polyunsaturated fatty acids such as

arachidonic acid and the acid Δ4,7,10,13,16,19-22:6 (DHA) accounting for 4.5 and 3.2 % respectively, have been identifed as N-acyl pyrrolidides.

DISCUSSION AND CONCLUSIONS

Typical Δ5,9 demospongic acids were detected in all sponges. The *Cinachyrella* sponges were associated with a number of bacteria. As demonstrated by electron microscopy, symbiotic bacteria were remarkably few in number in *Chalinula saudiensis*. They are extracellular and localised in a few areas of the mesohyl where collagen fibrils accumulate (VACELET *et al.,* 2001). Furthermore, the occurrence of Cyanobacteria was occasionally observed in this study. Thus, the unexpected occurrence of arachidonic acid and docosahexaenoic acid (DHA) may be attributed to the associated Cyanobacteria. Although electron microscopy studies on *Stylissa carteri* are still to perform, one can suggest that polyunsaturated fatty acids likely originate from Cyanobacteria. This work increases knowledge on biochemistry of the Red Sea sponges, including a new one.

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