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FATTY ACIDS, PHOSPHOLIPIDS, AND THE BETAINE LIPID DGTS FROM THE AQUATIC FERN *Salvinia natans*

O. A Rozentsvet,[1] T. Rezanka,[2] E. S. Bosenko,[1] E. A. Uzhametskaya,[1] and V. M. Dembitskii[3]

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Fatty acids, total lipids, phospholipids, and the betaine lipid DGTS in aerial and underwater leaves and sori of the aquatic fern Salvinia natans *were investigated. Lipid compositions from various parts (organs) of the fern were different. The distributions of polar lipids and fatty acids in various organs were discussed. Special attention was paid to the DGTS-forming capability of this fern.*

Key words: *Salvinia natans*, betaine lipid DGTS, fatty acids, phospholipids.

Despite the large number of fern-like plants, aqueous ferns of the Salvinia family number only ten species. They all grow in freshwater aquifers of tropical and subtropical countries, mainly in Africa and South America [1]. Only one species has adapted to life at temperature latitudes, *Salvinia natans* (L.) All. This species is distributed in Russia in the south and a central band of the European part, in western Siberia, and in the Far East [2].

Salvinia is a rare and disappearing plant that arrived during the preglacial period [3, 4]. The chemical composition of this fern is practically unstudied with the exception of the amino-acid composition [5].

The goal of the present work was to investigate the lipid compositions in cells of various tissue types of *Salvinia natans*. Aerial and underwater leaves and sorus were analyzed separately. Specimens were analyzed for total lipids (TL), qualitative and quantitive phospholipid (PL) composition, and quantitative betaine lipid 1,2-diacylglyceryl-3-O-4′-(N,N,N-trimethyl)homoserine (DGTS) content.

Table 1 gives the analytical results, which are average values from 2-3 parallel determinations of similar specimens (aerial and underwater leaves and sorus). It can be seen that the amount of TL in underwater leaves is almost twice that in aerial leaves but practically the same as that in sori. The total PL contents in aerial (462 μg/g dry wt.) and underwater leaves (451 μg/g dry wt.) are identical whereas that in sorus is twice as much (933 μg/g dry wt.).

The pure PL contained phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidyl- (PG) and diphosphatidylglycerine (DPG), phosphatidylinosite (PI), and phosphatidic acid (PA). The principal PL in aerial leaves was PC (40.5%) and then PG, PE, PA, and PI. We obtained analogous data by analyzing leaves from all terrestrial fern species [6, 7]. As before, PC was the highest of all PL in underwater leaves. However, its fraction was much greater than in aerial leaves and reached 71.7%. The PG fraction was only 2.7%. The amount of PC in sorus was 51.2%, i.e., almost the same as in aerial leaves. However, the PG content in them was analogous to that in underwater leaves (2.3%).

The contents of other PL also varied greatly. The PE content in underwater leaves was twice that in aerial leaves (21.0% vs. 10.8%). The DPG content in all investigated specimens was below the detection limit of the method used (<1%). This indicated that the lipid composition varied widely in cells from different tissues of a single plant. The PL distribution in aerial leaves was rather typical and characteristic of leaves from many higher plants [8] whereas the lipids from underwater leaves were richer in PC with a low PG content.

1) Institute of Volga Basin Ecology, Russian Academy of Sciences, 445003, Tol′yatti, Komzina-10, Russia, e-mail: rozen@infopac.ru; 2) Institute of Microbiology, Academy of Sciences of the Czech Republic, 142 20, Prague, Videnska 1083, Czech Republic; 3) Natural Lipid Group, Environmental Research Division, P. O. Box 39074, Hebrew University, Jerusalem, Israel. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 399-402, September-October, 2005. Original article submitted May 2, 2005.

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TABLE 1. Total Lipids, Phospholipids, and DGTS in Cells of Various Tissue Types of *Salvinia natans*

Lipids	Leaves		Sorus
	aerial	underwater	
Total lipids, mg/g dry wt.	14.0	27.1	13.5
Total phospholipids, μg/g dry wt.	462	451	933
Pure phospholipids:			
phosphatidylcholine	40.5	71.7	51.2
phosphatidylethanolamine	10.8	21.0	16.7
phosphatidylglycerol	32.4	2.7	2.3
phosphatidylinositol	8.1	5.2	20.2
phosphatidic acid	8.3	Tr.	9.5
DGTS, μg/g dry wt.	140	258	151

The betaine lipid DGTS, like PL, is a membrane structural component. Although PL are essential cell-membrane components of any organism, betaine lipids are found in a limited number of species [9-11]. The physiological role of these lipids has not yet been determined. However, they are some of the main lipids in many organisms. It has been demonstrated in several studies and reviews that the most often encountered betaine lipid DGTS was detected in lipids from microorganisms, fungi, algae, lichens, and in lower vascular plants but not in flowering plants [12-14]. This last feature suggests that this lipid has significance for evolution and, according to some authors, plays the same role in cells of lower plants as PC in higher plants [14]. The amount of DGTS in aerial leaves of the investigated fern was 140 μg/g dry wt. This was practically the same as the content of DGTS in sorus. The amount of DGTS in underwater leaves was greater at 258 μg/g dry wt.

The fatty-acid composition is an important characteristic from the viewpoint of membrane properties. Fatty acids impart fluidity, flexibility, and selective permeability to cell membranes. Table 2 lists the fatty-acid composition of lipids from aerial leaves and sori from GC—MS analysis. It can be seen that a rather wide range of fatty acids with hydrocarbon chain lengths from C-12 to C-30 was found in the lipid structure of the studied specimens. However, the main acids were the C-14—C-20 series. Sorus lipids were higher in saturated acids (52.52%) than leaf lipids (21.6%). The saturated acids were highest in palmitic acid (6.9% in leaves and 11.14% in sorus) and stearic acid (5.5% in leaves and 15.9% in sorus). However, saturated fatty acids from sorus lipids had a rather high content of C-20 (8.41%) and C-22 (4.68%) acids in addition to these acids.

Unsaturated fatty acids of lipids from leaves and sorus of *S. natans* included acids with various numbers of double bonds. The 16:1 (10.85%) and 18:1 (8.39%) acids dominated the monoene acids in leaf lipids whereas sorus lipids were highest in oleic acid (13.37%) with significant amounts of other monoene acids (for example, 20:1 acid was 2.34%; 24:1 acid, 2.19%). The total amount of monoene acids was greater in sorus lipids than in leaf lipids. The diene acids were mainly linoleic acid although it was observed only in leaves and not in sorus. Triene acids (16:3 and 18:3) were found in leaves and sori. However, there was five times more of them in leaf lipids than in sori lipids.

A characteristic feature of fatty acids from the studied fern was the presence of acids with a hydrocarbon chain length greater than 20 and high unsaturation. These acids were found only in leaf lipids. Thus, polyene acids of leaf lipids constituted almost a third of the total fatty-acid content.

Thus, different tissue types corresponding to different organs of a single fern species contained different ratios of membrane glycerolipids and different fatty-acid compositions.

Phylogenetic aspects of the distribution of membrane lipid components are discussed rather frequently in the literature. For example, the absence of DGTS in spermatic plants and its presence in many fern-like and green algae suggests that fern-like plants are more closely related to green algae than to spermatic plants with respect to this DGTS lipid [10, 15]. The relationship of these plant groups was confirmed because the fatty acids from both plant groups contained long-chain polyene acids such as arachidonic and eicosapentaeneoic, which are not synthesized by higher plants of the *Gymnospermae* and *Angiospermae* classes. Leaf lipids of *S. natans* also contained acids with chain lengths of 20 C atoms and more. Thus, on one hand ferns contain 18:3, 18:2, and 16:0 fatty acids, which are typical of spermatic plants. On the other, they also contain fatty acids typical of algae, 20:4 and polyunsaturated 20:5 acids. This is indicative of a position for fern-like plants that is intermediate between algae (lower) and spermatic (higher) plants.

TABLE 2. Fatty Acids of Lipids from *Salvinia natans*, %

Acid	Leaves	Sorus	Acid	Leaves	Sorus
12:0	0.85	0.66	22:1n-11	0.28	1.32
13:0	0.09	0.63	23:1	0.07	0.48
14:0	2.84	3.66	24:1n-13	0.19	2.19
15:0	2.14	3.06	25:1n-14	0.05	0.34
16:0	6.9	11.14	26:1n-15	0.06	1.24
17:0	0.92	1.29	27:1	0.02	0.36
18:0	5.5	15.9	28:1	-	1.06
19:0	0.20	0.32	30:1	-	0.85
20:0	1.16	8.41	$\Sigma_{monoene}$	27.44	37.26
21:0	0.14	0.42	14:2n-5	0.32	-
22:0	0.29	4.66	16:2n-4	0.59	-
23:0	0.13	0.36	18:2n-6	7.65	1.42
24:0	0.35	1.17	20:2n-6	0.33	-
26:0	0.07	0.64	22:2n-6	0.18	-
28:0	0.02	0.12	Σ_{diene}	9.07	1.42
30:0	-	0.08	16:3n-6	1.23	0.23
$\Sigma_{sat.}$	21.6	52.52	16:3n-3	1.41	0.42
12:1	0.55	0.27	18:3n-6	1.14	0.89
13:1	0.09	0.09	18:3n-3	5.55	0.96
14:1n-5	1.25	0.42	20:3n-9	0.36	-
15:1n-8	0.85	0.23	20:3n-6	0.53	-
15:1n-6	0.97	0.18	20:3n-3	0.65	-
16:1n-9	6.78	1.46	22:3n-6	0.32	-
16:1n-7	4.07	0.39	22:3n-3	0.44	-
trans-16:1n-13	0.16	-	24:3n-3	0.54	-
17;1n-10	0.37	0.21	26:3n-3	0.28	-
17:1n-8	0.22	0.47	Σ_{triene}	12.45	2.50
18:1n-11	1.33	2.02	16:4n-3	3.07	-
18:1n-9	5.83	13.37	18:4n-3	3.28	-
18:1n-7	1.23	3.19	20:4n-6	3.64	-
19:1n-10	0.27	0.59	20:4n-3	4.02	-
20:1n-13	0.17	0.55	20:5n-3	3.06	-
20:1n-11	0.61	1.73	22:4n-3	0.89	-
20:1n-9	1.06	2.34	22:5n-3	5.36	-
21:1n-12	0.11	0.26	22:6n-3	4.88	-
22:1n-15	0.17	0.17	$\Sigma_{polyene}$	28.20	-
22:1n-13	0.68	1.48	$\Sigma_{unknown}$	1.24	6.28

EXPERIMENTAL

GC—MS analysis of fatty acids was performed using the methyl esters in a Finnigan MAT spectrometer (Finnigan MAT, San Jose, CA) and a column (60 m × 0.25 mm, Supelcowax 10, 0.25 μm thick film; Supelco, Gland, Switzerland). The injector temperature was 100°C. The temperature program of the column was: 100°C, 1 min; 100-230°C, 7 min; 230-280°C, 25 min; 280°C, 10 min. The carrier gas was He at a flow rate of 70 cm/s. Spectra were scanned in the range *m*/*z* 60-700.

Plant Material. The aquatic fern *S. natans* (L.) All. (*Salviniaceae*, Order *Hydropteridales*, Class *Polypodiopsida*, Section *Polypodiophyta*) was investigated. Specimens of leaves and sori were collected in an unnamed freshwater aquifer in the Samara Bend Preserve in August 2001.

Lipid Extraction and Analysis. Lipids were extracted as before [16]. PL and DGTS were separated using thin-layer chromatography (TLC) on microplates (6×6 cm or 10×10 cm) with a fixed layer of silicasol (Haapsalu, Estonia). The solvent

systems $CHCl_3$:CH_3OH:C_6H_6:NH_4OH (130:60:20:12) in the first direction and $CHCl_3$:CH_3OH:C_6H_6:$(CH_3)_2CO$:CH_3CO_2H (140:60:20:10:8) in the second direction were used.

Lipids were identified by known methods using specific color reagents [17]. The amounts of PL were determined from the P content [18, 19]. DGTS was determined quantitatively by spectrophotometry after ashing in H_2SO_4. A calibration curve was constructed using known amounts of previously isolated and purified DGTS in the range 1-30 μg of DGTS [20].

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