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LIPIDS AND FATTY ACIDS FROM *Ulva intestinalis* FROM ESTUARIES OF THE CASPIAN BASIN (ELTON REGION)

O. A. Rozentsvet* **and V. N. Nesterov**

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The fatty-acid composition of total lipids from eurybiotic species of green alga Ulva intestinalis *from saltwater and estuaries of the Caspian arid zone basin (Elton Region) was studied. It was shown that the relative content of trienoic acids increased and the relative content of long-chain acids decreased as the salinity of the water increased. A feature of the phospholipid composition of* U. intestinalis *estuary populations was the presence of phosphatidylcholine in plants growing in water with salinity 10.0–11.6 g/L and its absence in algae at higher salinities (28.5–31.2 g/L).*

Keywords: *Ulva intestinalis*, estuaries, fatty acids, glycolipids, phospholipids, betaine lipid.

The multi-cellular macroalga *Ulva (=Enteromorpha) intestinalis* (L.) Link (Chlorophyta) is broadly distributed worldwide in littoral zones [1–3]. The lipid composition of *U. intestinalis* from marine habitats was studied in detail [4–7]. It was shown that glyco- (GL) and phospholipids (PL) comprised the main part of acyl lipids in lipids of this species from marine habitats. The principal PL was phosphatidylglycerol (PG). It was also established that membrane lipids from these algae contained the betaine ester lipid 1,2-diacylglycero-3-*O*-4′-(*N*,*N*,*N*-trimethyl)-homoserine (DGTS). A characteristic feature of *U. intestinalis* was the ability to synthesize in large quantities polyunsaturated fatty acids (PUFA) containing 16 and 18 C atoms in the hydrocarbon chain [8–10].

Eurybiotic alga species such as *U. intestinalis* are well adapted to habitation of marine waters, freshwater, salty water, and estuaries [1, 11]. The salinity of salty waters is considered to be 6–8 g/L; of estuaries, 18–30 g/L [12]. The salinity is one of the ecological factors that can affect many metabolic processes of hydrobiota such as the aquatic conditions, mineral exchange, metabolic processes, and; therefore, their growth, development, and distribution [13]. We found that *U. intestinalis* was one of the dominant species in rivers of the arid zone of the Russian northern Caspian basin [14].

The goal of the present work was to study features of the polar lipid and fatty acid (FA) composition of total lipids from *U. intestinalis* as a function of the habitat salinity. Samples of algae were collected in August 2010 in five small rivers, the salinity levels of which varied from 10.9 to 31.2 g/L (Table 1).

The content of total lipids in *U. intestinalis* cells increased with increasing salinity. In all instances the content of polar lipids was at the 89–95% level. The remainder was neutral lipids (NL) (Table 1). The results showed that the lipid profile of *U. intestinalis* polar lipids from highly saline rivers, like representatives of *Ulvales* from marine habitats, consisted of GL, PL, and DGTS. Mono- (MGDG) and digalactosyldiacylglycerins (DGDG) in addition to sulfoquinovosyldiacylglycerin (SQDG) were identified in the GL. The content of phosphorus-free lipid DGTS reached 4–6% of total lipids. In this respect *U. intestinalis* from estuaries did not differ from the same plants from marine habitats and from phylogenetic related species of the genus *Ulva* [7, 8, 11].

The PL fraction contained PG, phosphatidylethanolamine (PE), phosphatidylinositol (PI), diphosphatidylglycerin (DPG), and phosphatidic acid (PA). The principal P-containing lipid in a quantitative sense was PG (Table 2), the content of which reached 79% of total PL. Certain algae growing in water with salinity 10.9–11.6 g/L contained phosphatidylcholine (PC). The structural and functional significance, localization within the cell, and biosynthesis pathway of polar lipids are known to differ. According to current thinking, MGDG, DGDG, SQDG, and PG were the dominant structural elements of the chloroplast membranes; PL and DGTS, of non-plastid endomembranes [13]. If it is considered that PG was the dominant PL, then non-plastid membranes of *U. intestinalis* were constructed mainly of betaine lipids whereas PL for this species were minor components.

Institute of Volga Basin Ecology, Russian Academy of Sciences, 445003, Tol′yatti, Ul. Komzina, 10, fax: (8482) 48 95 04, e-mail: olgarozen@pochta.ru. Translated from *Khimiya Prirodnykh Soedinenii*, No. 4, July–August, 2012, pp. 492–495. Original article submitted January 11, 2012.

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TABLE 1. Composition of Total Lipids from *U. intestinalis* from Rivers with Various Salinities, % of Total

Lipids	Salinity, g/L				
	10.9	11.0	11.6	28.5	31.2
TL*	10.1	8.3	12.1	10.2	12.6
MGDG	38.1	38.8	38.7	36.6	35.4
DGDG	22.9	22.2	20.9	23.0	24.5
SQDG	22.5	25.9	18.9	22.3	24.3
DGTS	4.5	4.5	5.5	4.9	4.1
PL	4.0	3.7	4.8	6.2	4.2
NL	8.0	4.9	11.2	7.0	7.5

*TL in mg/g of raw mass.

TABLE 2. Composition of Phospholipids from *U. intestinalis* from Rivers with Various Salinities, % of Total

Lipids	Salinity, g/L				
	10.9	11.0	11.6	28.5	31.2
PC	5.5	3.0	4.5	0	0
PE	10.6	10.3	17.8	6.5	5.8
PG	66.2	72.1	64.1	72.3	79.0
PA	5.2	3.7	2.2	8.5	4.6
PI	11.4	10.5	11.0	11.9	10.1
DPG	1.1	0.4	0.4	0.8	0.5

The presence of betaine lipids in the polar lipids of green algae was an important point. The DGTS structures were reminiscent of PC and usually appeared when PC were absent or present in small quantities [15]. DGTS, PC, and PE were rather rarely encountered simultaneously [16]. For example, all three lipids were detected in eustigmatophytes of *Nannochloropcis* sp. [17] and *Monodus subterraneus* [18]. However, DGTS appeared more often with PC or PE. Thus, lipids of *Chlamidomonas reinhardtii* [19] contained PE and DGTS whereas both PC and DGTS were found in *Dunaliella salina* [20]. Our results showed that the PL of a single alga species can contain simultaneously the three aforementioned lipids and that PC can be present or absent (Table 2).

According to chromatographic analysis, the total lipids contained >30 FAs with chain length from 12 to 24 C atoms and with various degrees of unsaturation (Table 3). Greater than 90% of the total FAs consisted of C_{16}–C_{18} FAs. Palmitic acid $C_{16:0}$ dominated the saturated FAs. Trienoic and tetraenoic FAs contributed most to the unsaturated total lipids. The trienoic FAs consisted largely of α-linolenic acid, 24.1–28.8% of the total FAs and 90% of the total trienoates. γ-Linolenic acid ($18:3_{\omega6}$) (1.2–2.7%) was present in a smaller amount. The tetraenoic FAs were represented by $C_{16:4}$ (8.5–10.0%) and $C_{18:4}$ (11.2–18.1%). Both acids belong to the biosynthetic family of ω3 FAs.

The principal mono-unsaturated FAs were $C_{16:1}$ and $C_{18:1}$. The content of the former was 2.8–5.6%; of the latter, 7.8–9.7%. The octaenoic FAs had two isomers at the $C_{18:1\omega9}$ and $C_{18:1\omega7}$ positions. The dienoic acids were represented mainly by linoleic acid $C_{18:2}$, the content of which varied from 3.7 to 6.3%. Small amounts of $C_{16:2}$ and $C_{20:2}$ acids were also identified among FAs of several samples. The contribution of C_{20} PUFA to the total lipids was <6.5%. A small amount of FAs with short chains (1.2–2.9% of the total) was found. The contents of FAs with a branched chain and an uneven number of C atoms was <0.4%.

A comparison of FAs in lipids of *U. intestinalis* collected in rivers with different salinity levels showed that the ratio of saturated and unsaturated acids was practically the same depending on the salinity. However, a tendency toward increasing relative content of trienoic FAs ($r = 0.87$, $p \le 0.05$) and decreasing relative contribution of long-chain FAs ($r = -0.52$, $p \le 0.1$) with increasing salinity was seen.

The spectrum of FAs in *U. intestinalis* from river populations did not differ from that of marine representatives [7, 8, 10]. The principal PUFA were C_{16} and C_{18}. With respect to PUFA with long C chains, it is known that they are rather rare in green algae [7, 16]. Thus, specimens with a high content of $C_{20:3\omega6}$ were not found. Arachidonic acid ($C_{20:4\omega6}$) also was practically not detected in lipids from freshwater algae and did not exceed several percent of total FAs in marine specimens of green algae [8, 16]. Therefore, river populations of *U. intestinalis* did not differ from marine populations.

TABLE 3. Composition of Fatty Acids of Total Lipids from *Ulva intestinalis* from Rivers with Various Salinities, % of Total

Fatty acid	Salinity, g/L				
	10.9	11.0	11.6	28.5	31.2
12:0	0.1	0.2	0.1	0.1	0.1
14:0	2.6	1.0	1.1	0.9	0.6
14:1ω9	0.0	0.2	0.0	0.0	0.1
14:0-12-Met	0.0	0.2	0.3	0.4	0.1
15:0	0.2	0.0	0.0	0.0	0.3
16:0	17.9	20.9	22.8	22.4	19.8
16:1	5.6	2.8	2.8	3.5	3.9
16:2ω6	Tr.	Tr.	0.2	0.2	0.4
17:0	Tr.	Tr.	Tr.	Tr.	0.4
17:1ω9	0	0	0	0	0.1
16:3ω3	0.6	0.5	0.6	0.5	0.6
16:4ω3	10.0	12.5	8.5	10.0	9.5
18:0	0.9	0.9	0.9	0.8	0.6
18:1ω9	1.5	0.9	1.8	1.1	0.9
18:1ω7	8.2	6.9	7.5	6.7	7.3
18:2ω6	4.6	3.7	6.3	5.0	5.9
18:3ω6	2.7	1.5	1.2	1.8	1.6
18:3ω3	24.1	25.7	26.9	28.3	28.8
18:4ω3	14.4	18.1	11.2	13.2	13.6
20:0	0.1	0.2	0.2	0.2	0.2
20:1ω11	0.1	0.1	0.1	0.1	0.2
20:2ω9	0.1	0.2	0.2	0.2	0.2
20:3ω6	0.3	0.2	0.3	0.2	0.3
20:4ω6	1.2	0.4	1.1	1.2	1.2
20:4ω3	0.0	0.3	0.5	0.0	0.4
20:5ω3	2.5	0.8	0.9	1.0	0.7
22:0	0.3	0.5	0.3	0.5	0.6
22:4ω3	0.5	0.4	0.3	0.5	0.5
22:5ω3	1.5	2.0	1.1	1.4	1.2
24:0	Tr.	Tr.	Tr.	Tr.	0.1

Tr.: trace of FA, the content of which was <0.05% of the total.

The concentration and composition of salts in water can affect various metabolic processes of algae [13]. The optimum salinity is different for different alga species. The salinity in the studied rivers of Elton Region differed by greater than three times. However, a clear dependence of the salinity on the amount of total lipids was not found. Apparently the salinity was not the only factor affecting the amount of total lipids in algae in river waters.

The results showed that the FA composition of river populations of *U. intestinalis* did not differ from that of marine populations. However, the compositions of polar lipids from algae of estuaries and salty rivers differed from that of the same algae inhabiting marine waters. The differences were related to the absence of PC in PL of *U. intestinalis* growing in rivers in which the salinity was practically equal to that in marine waters and its presence in algae inhabiting freshwaters (salinity <11.6 g/L). The appearance of this lipid in algae growing in waters with lower salt concentrations could be considered as a manifestation of the adaptation of this eurybiotic species to various salinities.

EXPERIMENTAL

Specimens of *Ulva (=Enteromorpha) intesinalis* (L.) Link (Ulvales, Ulvaceae) [21, 22] were collected in small rivers and the arid zone of the northern Caspian basin of Russia. The salinity levels were Khara River (49°12′ N, 46°39′ E) 11 g/L; Lantsug River (49°12′ N, 46°38′ E), 11.6; Smaragda River (49°07′ N, 46°47′ E), 10.9; Solyanka River (49°10′ N, 46°35′ E), 28.5; and Chernavka River (49°12′ N, 46°40′ E), 31.5.

Samples were preserved in formalin solution in order to determine the species. Three independent biological samples (2–4 g of raw mass) were taken from each part of the selected material. Deproteination was carried out in *i*-PrOH (5 mL) at 80°C for 15 min. The processed material was supplied in tightly sealed glass vials to the analytical lab.

Lipids were extracted using $CHCl_3$:MeOH (1:2, v/v) by the literature method [23]. Lipids were separated into individual classes by TLC on plates (6 × 6 cm or 10 × 10 cm) with a fixed layer of silica gel (Haapsalu, Estonia). GL were separated using acetone:C_6H_6:H_2O (91:30:8); PL, $CHCl_3$:MeOH:C_6H_6:NH_4OH (130:60:20:12) in the first direction and $CHCl_3$:MeOH:C_6H_6:Me_2CO:HOAc (140:60:20:10:8), in the second.

Lipids were identified using standards and specific reagents for individual functional groups [24]. Lipids were detected after separation by spraying with H_2SO_4 (10%) in MeOH. The amount of PL was determined spectrophotometrically from the inorganic P content [25]; of GL, by densitometry. A calibration curve was constructed using MGDG (Larodan, Sweden). The DGTS content was determined spectrophotometrically. A calibration curve for DGTS determination was constructed from DGTS that was isolated and purified beforehand in the range 1–10 μg.

FAs were analyzed as methyl esters prepared by refluxing in HCl solution (5%) in MeOH for 1 h. Esters were purified by preparative TLC and analyzed on a Chromatek Kristall 5000.1 GC (Russia) using a capillary column (105 m × 0.25 mm diameter, Restek, USA) and isothermal conditions. The column temperature was 180°C; vaporizer and detector, 260°C. The carrier gas (He) flow rate was 2 mL/min. Peaks were identified by comparison of retention times with those of standards (Sigma, USA).

The tables present averages of 2–3 biological repetitions for 4–6 analytical determinations. The relative standard deviation for lipids and dominant FAs was 2–5% and <10% for the minor components. Differences were assessed using the Student criterion for significance level $p < 0.05$.

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