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LIPIDS FROM THE MARINE WORM *Urechis unicinctus*

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The lipid composition of four tissues, musculo-cutaneous, blood, internal organs, and gonads, from the marine worm Urechis unicinctus *was studied. It was shown that the principal phospholipids (PL) in the studied tissues were phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Depending on the tissue type, the fraction of PC was 31.8–42.6%; PE, 16.1–25.7% (of total PL). Blood contained a high percentage of lyso-PE (20.8%). The fatty-acid (FA) composition of the tissues consisted of C16, C18, C20, and C22 families with different degrees of unsaturation. Eicosapentaenoic acid (20:5) had the highest percentage in all tissues at 15.5–26.1%. Tissues with different functions such as musculo-cutaneous and gonads contained the same amounts of 20:5 FA, 26.1% (of total FA). The high content of polyunsaturated FA and, in particular, eicosapentaenoic FA in* U. unicinctus *confirmed that it was valuable as not only an energy source but also a source of PL and essential FA.*

Keywords: fatty acids, marine worm, *Urechis unicinctus*, phospholipids.

Worms are some of the least studied among the large variety of marine organisms. Marine worms are an important link in the food chain. For example, *Urechis unicinctus* is a staple of aboriginal people of the Far East and a food source for commercial fish. The goal of our study was to determine the composition and content of lipids from tissues of *U. unicinctus* such as musculo-cutaneous (MC), blood, internal organs, and gonads.

Total lipids in these tissues of the marine worm (MW) were 5.9, 14.8, 34.9, and 37.4% (per dry tissue weight), respectively. Analysis of the neutral lipids (NL) showed that triacylglycerols and cholesterol were the main ones in all studied tissues. Pigments and glycerin alkyl esters were also present. In addition, internal organs and blood contained fatty acid methyl esters (FAME); MC and blood, traces of 1,3-diacylglycerols. According to TLC, blood had the greatest variety of NL. Isay et al. determined earlier the content in worms, including *U. unicinctus*, of α-glyceryl ethers (α-GE), derivatives of glycerin with an ether bond [1]. An analysis of six MW species showed that the α-GE content in them varied in the range 1.9–4.0% (in the lipid extract); the α-GE content in *U. unicinctus*, 2.2%. Marsden obtained data indicating that not all four studied species of tropical MW contained GE (TLC data) [2].

Table 1 presents the composition and content of PL from the tissues. The main ones were phosphatidylcholine (PC) and phosphatidylethanolamine (PE). This agreed with the literature data [3]. The PL content in three species of MW was reported [4]. The PL fraction in the lipid extract varied markedly from species to species (21.2–51.0%). Phosphatidylglycerin (PG) was detected in *U. unicinctus* tissues studied by us only in traces in blood. Sphingomyelin (SM) was found in three tissues and was absent in blood. As it turned out, the fraction of lyso-PE was significant in blood, up to 21%, whereas lyso-PC was found only in gonads and MC in insignificant (<1%) quantities. The disparity in the literature data on the composition of particular PL and their content is a function of many factors, including the type of extraction. Thus, Katrich and Isai showed [5] that the SM content, like certain other PL, increased markedly upon replacing the traditionally used Folch and Bligh–Dyer extraction methods [6, 7]. It is thought that PL play the main role in various cell processes and, therefore, are important factors in cell disfunction and diseases [8–12]. One of the principal discoveries in lipid biology over the last 10 years was the detection of the broad physiological activity of lyso-PL [13].

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TABLE 1. Phospholipid Content in *U. unicinctus* Tissues (% Relative to Total Phospholipids)

PL	Tissue type			
	blood	gonads	internal organs	musculo-cutaneous
PC	42.4	42.6	31.8	38.7
PE	18.1	18.1	25.7	16.1
LPE	20.8	4.5	6.1	8.9
SM	–	0.4	3.3	0.6
PI	0.45	7.2	2.5	4.1
LPC	–	0.3	–	0.4
PS	0.3	12.3	12.6	11.7
DPG	4.5	11.8	4.8	0.4

TABLE 2. Fatty-Acid Content in *U. unicinctus* Tissues (% of ΣFA)

FA	Musculo-cutaneous	Blood	Internal organs	Gonads
14:0	2.46	2.93	4.43	2.61
14:1 n-11	–	–	–	1.21
15:0	0.42	Tr.	0.76	Tr.
15:1n-11	Tr.	–	Tr.	–
16:0	9.71	16.51	12.96	17.53
16:1n-11	2.74	10.51	11.61	8.81
16:3n-3	–	–	1.10	–
16:4n-1	–	–	2.33	–
17:0	0.94	0.94	0.82	Tr.
18:0	7.45	6.69	5.89	4.64
18:1n-11	1.44	4.56	2.52	3.56
18:1n-9	7.11	14.80	7.38	10.02
18:2n-6	Tr.	Tr.	0.42	Tr.
18:4n-3	Tr.	Tr.	2.15	Tr.
19:1n-11	–	–	–	3.04
19:1n-6	–	–	–	2.15
20:1n-11	12.20	8.02	7.57	6.80
20:1n-6	3.42	3.09	2.82	1.31
20:2n-11	Tr.	0.78	Tr.	Tr.
20:2n-6	3.20	3.12	2.60	2.30
20:4n-3	2.21	Tr.	1.36	0.60
20:5n-3	26.11	15.53	22.68	26.07
21:5n-3	0.94	Tr.	Tr.	Tr.
22:2n-11	4.45	1.85	1.04	0.40
22:2n-6	6.56	3.95	2.03	1.91
22:3n-6	0.55	–	–	–
22:4n-3	0.87	2.11	0.68	–
22:5n-3	3.04	Tr.	1.43	0.92
22:6n-3	3.59	2.99	4.04	4.14
X	0.58	1.60	1.31	1.97
Saturated	21.00	27.10	24.90	24.80
Unsaturated	78.40	70.53	73.77	73.23
Monoenoic	26.90	40.20	31.90	36.90
Dienoic	14.20	9.70	6.10	4.60
Trienoic	0.55	–	1.10	–
Tetraenoic	3.08	2.11	6.52	0.60
Pentaenoic	30.08	15.53	24.11	26.99
Hexaenoic	3.59	2.99	4.04	4.14

Tr., trace; dashes in columns indicate the particular acid is absent.

We also studied the composition and content of fatty acids (FA) in *U. unicinctus* tissues (Table 2) because current medical hypotheses associate many human diseases with an *in vivo* deficit of polyunsaturated FA (PUFA). Many researchers propose alleviating the PUFA deficit using diet [14–18] or drugs containing PUFA [19, 20]. Several researchers consider diet to be important in reducing the risk of cancer. Thus, Bidoli et al. associated the inhibition of kidney cancer with a diet rich in PUFA [21].

Our results for FA in *U. unicinctus* showed (Table 2) that the predominant PUFA was 20:5 (eicosapentaenoic acid, EPA). Its contents in various organs such as MC and gonads were the same (26%). It is thought that tissues such as brain, retina, and reproductive organs are especially rich in *n*-3 PUFA [22].

Our data for *U. unicinctus* were similar to those of Kharlamenko et al. for docosahexaenoic PUFA (22:6) from *Chaetopterus cautis* [23]. It is known that the collection time (season) of an organism has an important influence on the FA content and that the PUFA content differs markedly from species to species. Thus, arachidonic (20:4) PUFA was predominant in *U. unicinctus* (April collection) in a previous study by Isai and Busarova [24]. Its fraction in total FA was 22.6%. EPA was not found. However, our previous data [24] for another marine worm species *Chaetopterus variopedatus* were practically identical to those of Kharlamenko et al. [23] for *C. cautis* for PUFA such as 20:4 (5.2%), 20:5 (13.7%), and 22:6 (10.7%). The high EPA content in *U. unicinctus* confirmed its food value for fish and man. The influence of EPA on diseases such as thrombosis [19, 25, 26], cancer [16, 21, 27], and other chronic diseases [17, 23, 28] has been studied.

EXPERIMENTAL

Study Subjects. Four specimens of *U. unicinctus* were caught at the Marine Experimental Station (Peter the Great Bay, Sea of Japan) in July. The total mass was 409.5 g. The tissues MC, blood, internal organs, and gonads were analyzed. The weights of the studied organs (relative to worm weight) were 33.8, 42.5, 16.3, and 7.4%, respectively.

Lipid Extraction. The extraction was performed using the Folch [6] and Bligh–Dyer [7] methods but with MeOH replaced by EtOH. Tissue was ground. An average sample (20 g) was taken for the extraction, treated with $CHCl_3$:EtOH (1:2 v/v, 60 mL), diluted with $CHCl_3$ (20 mL), and homogenized for 30 s. The supernatant was decanted. The solid was re-extracted with $CHCl_3$:EtOH (2:1 v/v, 6–7 volumes). The homogenate was left for 30 min and centrifuged. The extract was treated with H_2O (20%). After the phases separated, the $CHCl_3$ layer was evaporated to dryness in vacuo. Lipids were dissolved in $CHCl_3$.

Analysis of NL. The analysis was performed by TLC using Sorbfil plates with STKh-1A silica gel and glass plates with silica gel affixed by gypsum. Plates were activated in a drying oven for about an hour at 110°C before performing TLC. We used solvent systems [29] petroleum ether:Et_2O:HOAc (82:18:1; 90:10:1; 80:20:1) and heptane:benzene (9:1) with detection by H_2SO_4 (10%) in EtOH.

PL analysis was carried out using 2D micro-TLC on Sorbfil plates with STKh-1A silica gel and glass plates with silica gel affixed by gypsum. Plates were activated in a drying oven for about an hour at 110°C before performing TLC. We used solvent systems (first direction) $CHCl_3$:MeOH:C_6H_6:NH_4OH (60:30:10:6); $CHCl_3$:MeOH:NH_4OH (28%) (65:25:5); $CHCl_3$:Me_2CO:MeOH:HCO_2H:H_2O (100:40:20:20:8); (second direction) $CHCl_3$:MeOH:C_6H_6:Me_2CO:CH_3CO_2H:H_2O (70:30:10:5:4:1) [30], $CHCl_3$:Me_2CO:MeOH:CH_3CO_2H:H_2O (6:8:2:2:1), and Me_2CO:C_6H_6:HCO_2H:H_2O (200:30:3:10) [31]. Lipids on chromatograms were detected by H_2SO_4 solution (10%) in EtOH [32]. Specific reagents for detecting PL were a molybdate reagent [33]; compounds with a free amine, ninhydrin solution (0.2%) in Me_2CO. Choline-containing and other compounds with quaternary ammonium groups were detected using Dragendorff's reagent [29].

Quantitative Determination of PL [29]. The amount of lipids in the extract was determined gravimetrically. For this, the content of phosphorus in aliquots of the extract or silica-gel bands from plates containing PL were analyzed in extracts or separate PL classes after separation by micro-TLC. The standard for constructing a calibration curve was mono-substituted potassium phosphate consisting of 1 μg P in 5 mL of final volume (0.1 μg per 0.5 mL) that gave an absorption of 0.175–0.180 optical units at 815 nm. Measurements were made on a CECIL Instruments spectrophotometer (England).

Fatty acid methyl esters (FAME) were prepared by the Hartman method [34]. FAME were analyzed as follows. FAME were purified by TLC (6 × 6 cm plates with silica gel affixed by gypsum) using benzene before the GC analysis. The purified fraction was analyzed on a Hewlett–Packard Model 6890 chromatograph using an HP-5-MS-5% capillary column (phenylmethylsiloxane) (30 m × 250 μm × 0.25 μm); Ar carrier gas, t = 220°C, isothermal. GC–MS was carried out on an HPGC/MSD 5393, HP-5MS with a capillary column programmed at 3°C/min from 140–280°C using the US NBS (mass spectra database).

REFERENCES

1. S. V. Isay, M. A. Makarchenko, and V. E. Vaskovsky, *Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol.*, **55**, 301 (1976).
2. J. R. Marsden, *Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol.*, **53**, 225 (1976).
3. E. Ya. Kostetskii and P. V. Velanskii, *Biol. Morya (Vladivostok)*, **34**, 103 (2008).
4. V. E. Vaskovsky and E. Y. Kostetsky, *Chem. Phys. Lipids*, **3**, 102 (1969).
5. E. M. Katrich, S. V. Isai, and T. Ya. Zvyagintseva, *Khim. Prir. Soedin.*, 205 (1993).
6. J. Folch, M. Lees, and G. U. Sloone-Stanley, *J. Biol. Chem.*, **226**, 497 (1957).
7. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.*, **37**, 911 (1959).
8. M. Bogdanov, E. Mileykovskaya, and W. Dowhan, *Lipids in Health and Disease. Subcellular Biochemistry, Phospholipids*, Vol. 49, Springer, Netherlands, 2008, p. 195.
9. J.-P. Mattila, K. Sabatini, and P. K. Kinnunen, *Biophys. J.*, **93**, 3105 (2007).
10. J. E. Vance, *J. Lipid Res.*, **49**, 1377 (2008).
11. Y. A. Wang, S. K. Johnson, B. L. Brown, L. M. McCarragher, K. Al-Sakkaf, J. A. Royds, and P. R. M. Dobson, *Int. J. Cancer*, **123**, 1536 (2008).
12. J. A. Berliner, N. Leitenger, and S. Tsimikas, *J. Lipid Res., April Supplement*, 207 (2009).
13. P. J. Quinn and X. Wang, *Lipids in Health and Disease. Subcellular Biochemistry, Phospholipids*, Vol. 49, Springer, Netherlands, 2008, p. 268.
14. R. G. Ackman, *Nutritional Evaluation of Long-Chain Fatty Acids in Fish Oil*, Academic Press, London-New York, 1982, p. 25.
15. A. P. Simopoulos, R. R. Kifer, and R. E. Martin, *Health Effects of Polyunsaturated Fatty Acids in Seafoods*, Academic Press, Orlando-San Diego-New York-Austin-Boston-London-Sydney-Tokyo-Toronto, 1986.
16. K. K. Carroll, *Health Effects of Fish and Fish Oils*, ARTS Biomedical Pub. and Distributors, St. John's, Newfoundland, Canada, 1989, p. 395.
17. A. P. Simopoulos, *Am. J. Clin. Nutr.*, **70**, 560S (1999).
18. J. X. Kang and K. H. Weilandt, *Lipids in Health and Disease*, Vol. 49, Springer, Netherlands, 2008, p. 133.
19. J. Hladovec and F. Kornalic*, Thromb. Res.*, **58**, 505 (1990).
20. P. Calder, in: *Food and Nutritional Supplements. Their Role in Health and Disease*, J. K. Ransley, J. K. Donnelly, and N. W. Read (eds.), Springer, Berlin, Heidelberg, New York, 2001, p. 275.
21. E. Bidoli, R. Talamini, A. Zuccherro, J. Polesel, C. Boseti, E. Negri, D. Maruzzi, M. Montella, S. Franceschi, and C. La Vecchia, *Int. J. Cancer*, **122**, 2586 (2008).
22. A. Nordoy, *Drugs*, **42**, 331 (1991).
23. V. I. Kharlamenko, S. I. Kiyashko, S. A. Rod′kina, and A. B. Imbs, *Biol. Morya (Vladivostok)*, **34**, 115 (2008).
24. S. V. Isay and N. G. Busarova, *Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol.*, **77**, 803 (1984).
25. K. A. Jorgensen and J. Dyerberg, *Dan. Med. Bull.*, **27**, 253 (1980).
26. Y. Nagakawa, H. Orimo, M. Harasawa, I. Morita, K. Yashiro, and S. Murota, *Atherosclerosis*, **47**, 71 (1983).
27. M. E. Begin, U. N. Das, G. Ells, and D. F. Horrobin, *Prostaglandins Leukotrienes Med.*, **19**, 177 (1985).
28. M. Yokoyama, H. Origasa, M. Matsuzaki, Y. Matsuzawa, Y. Saito, Y. Ishikawa, S. Oikawa, J. Sasaki, H. Hishida, H. Itakura, T. Kita, A. Kitabatake, N. Nakaya, T. Sakata, K. Shimada, and K. Shirato, *Lancet*, **369**, 1090 (2007).
29. M. Kates, *Techniques of Lipidology: Isolation, Analysis, and Identification of Lipids*, Elsevier, New York, 1972.
30. V. E. Vaskovsky and T. A. Terekhova, *J. High Resolut. Chromatogr.*, **2**, 671 (1979).
31. S. V. Khotimchenko, N. G. Klochkova, and V. E. Vaskovsky, *Biochem. Syst. Ecol.*, **18**, 93 (1990).
32. V. E. Vaskovsky, E. Y. Kostetsky, and I. N. Vasendin, *J. Chromatogr.*, **114**, 129 (1975).
33. V. E. Vaskovsky and N. A. Latyshev, *J. Chromatogr.*, **115**, 246 (1975).
34. L. Hartman, C. A. Lago, and N. Regino, *Lab. Pract.*, **22**, 475 (1973).