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[COMMUNICATION]

Phospholipids and Fatty Acids in Intact and Regenerating *Dugesia anceps*, a Fresh Water Planaria

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ABSTRACT—Phospholipids and their fatty acid composition in intact and regenerating *Dugesia anceps*, a fresh water planaria, were determined. Phospholipids and their percentual distribution in whole animals, in regenerating trunks and heads were similar. A decrease in the percentual contribution of several unsaturated fatty acids in phospholipids of regenerating animals was observed.

INTRODUCTION

The regenerative power of fresh water planarians has been known for more than 150 years [1]. Some morphological and cytological aspects of regeneration in these animals are well known. The process has been studied morphologically at the ultrastructural level [2]. From a biochemical point of view, the role of dopamine, serotonin and cAMP in regeneration has been determined [3], but no similar studies were conducted on lipids. This work is centered on the phospholipids content and their fatty acid composition in intact and regenerating *D. anceps*, a fresh water planaria; to our knowledge, this is the first report on this subject.

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MATERIALS AND METHODS

Animals The animals used were collected in Arroyo Naposta, a stream in the vicinity of Bahia Blanca. The determination of the species was reported in [4]. The specimens were kept in glass containers filled with filtered water from the same stream at room temperature [5]. They were fed fresh raw bovine liver and subsequently fasted for six days before use.

Regeneration For regeneration experiments, the heads of a set of about 120 animals were amputated just before the auricles; several subsets of heads and trunks were kept in separate containers filled with food-free filtered stream water. For analytical purposes they were retrieved after varying intervals (from four to five days in the case of heads and four to seven days in the case of trunks) but always before completion of regeneration. Intact planarians were simultaneously kept to be used as control. The control was performed either with whole animals or with heads and trunks sectioned just before homogenizing.

Lipid extraction After several washings with distilled water, planarians, whether control or regenerating, were transferred to a mortar. Lipids were extracted by homogenizing the tissue with 3 ml chloroform/methanol (2 : 1, v/v) [6]. The crude

total lipid was washed with $CaCl_2$ 0.05%, dried under N_2 and resuspended in a small volume of solvent. Samples were stored at −80°C under N_2.

Phosphoglyceride separation Phospholipids were isolated by two dimensional chromatography in 250 μm thick silica gel H plates (Merck-Sharp and Domme) following the procedure of Rouser *et al.* [7]. After development with either I_2 vapor or dichlorofluorescein (0.2%) in methanol, phospholipids were scraped into screw-capped tubes.

Phospholipid phosphorus determination Phosphorus was measured by the procedure of Rouser *et al.* [7] after perchloric acid digestion.

Methanolysis and gas liquid chromatography It was done with BF_3 in methanol according to Morrison and Smith [8]. Fatty acid methyl esters were analyzed in a model 3700 Varian gas liquid chromatographer, equipped with a hydrogen flame detector; a stainless steel column (2 m×2 mm ID) packed with OV 275 on N_2 carrier was used. Samples were run with temperature programmed between 160°C and 220°, 5°C/min, with the injector and the detector maintained at 220°C and 240°C, respectively. The measurement of peak areas was done with an automatic integrator (CDS-111) attached to the GLC. Fatty acids were identified by comparing their retention times with those of standard methyl esters.

Quantitative Analysis Quantitative determinations were made in triplicate samples from at least 30 trunks, heads or whole planarians and are expressed as mean ± standard deviations. Significance was determined by using the two tailed Student's *t*-test.

Unsaturation index This index is defined as the percentage of the sum of the product of the number of double bonds in each fatty acid methyl ester times its percentage.

RESULTS AND DISCUSSION

Phospholipid composition of intact and regenerating planarian tissues

The phospholipid composition of regenerating heads and trunks and of control animals is shown in Table 1. No significant differences could be found between them. PC and PE amount to more than 80% of the total, acidic phospholipids (PS and PI) to about 10%, the precursor PA to less than 1% and phosphonolipids to approximately 2%.

Although some mechanisms controlling regeneration in planaria have been reported [3], the possible, if any, role of lipids during that process is unknown. The results shown in Table 1 indicate that no differences in lipid composition exist between the regenerate and the old tissue.

Table 1. Phospholipid composition of intact and regenerating planarian tissues

Phospho lipids	Heads		Trunks		Whole Animals
	C	R	C	R	C
PC	50.8±2.1	52.0±3.8	55.8±8.0	53.4±9.2	49.0±3.5
PE	34.9±1.5	35.0±0.8	33.0±7.9	32.0±6.6	36.6±4.5
PS	7.8±1.2	7.2±1.0	4.5±3.1	5.6±1.6	6.6±0.7
PI	3.5±1.0	3.3±1.2	4.2±1.4	4.4±1.1	4.5±1.6
PA	0.3±0.2	0.4±0.0	0.4±0.1	0.8±0.6	0.3±0.2
CL	0.3±0.2	0.2±0.1	0.2±0.0	0.6±0.6	0.7±0.4
L	0.0±0.0	0.1±0.1	0.0±0.0	0.2±0.1	0.2±0.2
PNL	2.4±0.8	1.8±1.4	1.9±1.6	3.0±1.1	2.1±0.7

PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; PS, phosphatidylserine; PI, Phosphatidylinositol; PA, Phosphatidic acid; CL, Cardiolipin; L, Lysophospholipids; PNL, phosphonolipids; C, Control; R, Regenerating. Mean percentages ±SD of total phospholipid phosphorous from 3 samples of 30–120 pooled planarians.

TABLE 2. Fatty acid distribution in phospholipids of control and 4-day regenerating planarian trunks

FA	PC		PE		PS		PI	
	C	R	C	R	C	R	C	R
14:0	0.2±0.1	0.3±0.1	0.2±0.2	0.5±0.2	0.0±0.0	0.3±0.3	0.0±0.0	0.2±0.2
15:0	0.3±0.2	0.2±0.1	0.9±0.3	0.5±0.3	0.0±0.0	0.2±0.2	0.0±0.0	0.3±0.3
15:1	0.5±0.2	0.4±0.1	2.8±0.5	0.1±0.1**	0.1±0.1	0.2±0.1	0.1±0.1	0.1±0.1
16:0	16.0±1.5	18.0±0.9	11.3±0.7	12.4±0.7	2.6±0.6	3.8±0.5	1.0±0.5	2.6±0.6*
16:1	1.7±0.2	1.0±0.3*	7.5±0.9	3.6±0.6**	1.2±0.5	1.4±0.6	0.6±0.3	0.7±0.3
18:0	11.7±0.8	11.5±0.9	16.0±1.4	19.5±2.3	40.0±3.0	44.2±2.9	42.5±0.5	45.2±3.1
18:1	35.4±0.9	35.6±0.9	14.0±1.0	14.3±2.1	13.1±2.0	15.0±1.5	19.0±2.6	21.7±3.0
18:2	4.8±0.9	4.9±0.3	4.2±0.8	3.6±0.6	3.0±0.6	3.9±0.6	6.9±2.0	8.0±0.8
20:1	1.9±0.5	2.0±0.4	2.3±0.4	0.8±0.3**	2.2±0.4	0.6±0.3**	1.5±0.4	0.6±0.3*
20:3n6	1.2±0.4	1.3±0.6	1.5±0.6	1.6±0.2	1.2±0.4	1.1±0.8	1.5±0.4	0.9±0.3
20:4n6	8.0±0.6	9.3±1.4	5.2±0.6	6.2±0.5	9.8±1.2	11.5±0.9	17.5±0.6	10.6±1.5**
20:5n3	3.0±0.3	1.9±0.3*	1.1±0.4	1.2±0.3	2.3±0.5	1.4±0.4	2.5±0.3	1.1±0.3**
22:5n3	11.5±1.4	11.3±0.8	26.0±0.9	26.9±2.5	18.2±0.9	10.5±0.8**	4.3±0.3*	2.0±0.5**
22:5n6	2.6±0.3	2.2±0.4	6.0±0.5	7.4±1.2	5.6±0.2	5.2±0.5	2.1±0.3	5.4±0.7**
22:6n3	1.2±0.2	0.1±0.1**	1.0±0.5	1.4±0.5	0.7±0.4	0.7±0.3	0.5±0.2	0.6±0.3

The data represent the results of three samples of 30–120 pooled trunks. The n indicates the double bonds from the methyl end of the acid chain. Other abbreviations as in Table 1. *, statistically significant difference ($P<0.05$); **, statistically very significant difference ($P<0.01$).

The presence of phosphonolipids has been reported in ciliates, Cnidaria [9] and Mollusca [10]. In all these groups. as in planaria, epidermal cells are not protected by a cuticula and they are directly exposed to the medium [11]. The chemical properties of phosphonolipids could help these animals to cope with environmental changes [11, 12]. Conversely, phosphonolipids are absent in *Taenia* [13] which although being also a member of the phylum Platyhelmintes, is protected from external damage by a very active syncyctial epidermis. SPH was not present although large amounts of it have been reported in several invertebrate species [14, 15].

Fatty acid composition and changes during regeneration

Information about fatty acid composition of phospholipids in invertebrates has been growing in the last decade [16]. The activity of protein kinase C, apparently involved in planarian regeneration, as well as some functional properties, like for instance membrane fluidity, depend on that composition [3, 17, 18].

Fatty acid composition of PC, PE and acidic phospholipids was compared in control trunks and in regenerating trunks in the fourth day of the process (Table 2). Saturated fatty acids amount approximately to 30% in PC and PE and to 44% in PS and PI.

At that stage of the regeneration process, phospholipids undergo changes in their percentual composition. The table is basically a 15×8 matrix showing the 60 possible differences between control and regenerating animals. Four of the differences were found to be statistically significant ($P<0.05$) and ten statistically very significant ($P<0.01$). Two of them are increases (both occurring in PI) and twelve are decreases. These changes caused the decrease in the unsaturation index in PS and PI from 2% to 1.6% and from 1.6% to 1.3%, respectively. The more important variations are thouse of 20:4n6 in PI and 22:5n3 in PS, since these fatty acids are major constituents of these phospholipids.

20:5 has been found in most phospholipids in invertebrates [15, 16, 19]. In agreement with these reports, it was consistently found here in all phospolipids analyzed, although it represented only a small fraction. Curiously, this fatty acid

along with other unsaturated fatty acids decreased its percentual contribution in several phospholipids during regeneration. The polyene 22 : 5n3 in PI also follows the general pattern although its decrease is partially compensated by an increase in 22 : 5n6. The percentrual participation of 16 : 0 and 22 : 5n6, which increased in PI during regeneration, is at odds with the pattern found in the rest of the fatty acids. The fact that many variations were statistically non significant does not allow to conjecture about the fate of the fatty acids whose contribution diminished during regeneration.

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