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Regulation of membrane lipid bilayer structure during seasonal variation: a study on the brain membranes of *Clarias batrachus*

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Abstract

(1) A significant seasonal variation in the membrane fluidity (as sensed by DPH-fluorescence polarization), membrane lipid components (phospholipid and neutral lipid), fatty acid composition of membrane phospholipid (phosphatidylcholine, phosphatidylethanolamine and sphingomyelin), positional distribution of fatty acids at *Sn*-1 and *Sn*-2 position of phosphatidyl-choline and -ethanolamine is noticed in the brain membranes (myelin, synaptosomes, and mitochondria) of a tropical air breathing teleost, *Clarias batrachus*. (2) A 'partial compensation' of membrane fluidity during seasonal adaptation is observed in myelin and mitochondria membrane fractions. Synaptosomes membrane fraction exhibits a different response. Depletion (about 15–70%) of membrane lipid components (phospholipid, cholesterol, diacylglycerol and triacylglycerol) per unit of membrane protein is the characteristic feature of summer adaptation. An increase (about 20–100%) in the level of oleic acid and decrease (about 20–60%) in the level of stearic acid are almost common features in membrane phospholipid fractions of winter-adapted *Clarias*. (3) From the tissue slice experiment it is evident that there is an activation of cellular phopholipase A_2 at lower growth temperature and of cellular phosphalipase A_1 at higher growth temperature and this suggests the reorganization of molecular architecture of the membrane during seasonal adaptation. (4) Accumulation of oleic acid in *Sn*-1 position and polyunsaturated fatty acids in *Sn*-2 position of phophatidyl-choline and -ethanolamine during winter indicates an increase in the concentration of 1-monoenoic, 2-polyenoic molecular species of phospholipid in order to maintain the stability of membrane lipid bilayer.

Keywords: Membrane; Fatty acid; Phospholipid; Fluidity; Seasonal adaptation; Lipid; Lipid mediator; Glycolipid

1. Introduction

In the absence of thermoregulation, the homeostatic maintenance of physiological functions in poikilothermic animals necessitates adjustments in both the rate of biochemical reactions and the structural composition of the bio-molecules [1]. Perhaps the most significant adaptation in poikilothermic animals to changes in environmental or ambient temperature is the regulation of membrane microviscosity or fluidity, which is known as homeoviscous adaptation. This term was first coined by Sinensky [2] for *Escherichia coli* grown at 15° and 43°C. Considering

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the fluid mosaic model of cell membrane [3], the membrane lipid bilayer, which itself is very sensitive to environmental perturbations, regulates the dynamic state of membrane and thus monitoring the physiological functions and all other membrane-associated cellular activities. Although a parameter-like fluidity may not be adequate to explain the role of membrane lipid bilayer in maintaining proper membrane function, the concept of homeoviscous adaptation in its broad context, viz., the regulation of membrane function by modulation of lipid composition, remains a useful one [4].

Adaptation of membrane fatty acid composition and the physical properties to temperature by fish liver [5–8], lymphocytes [9], fish brain [10–13], fish intestine [14], frog epidermal cells [15] and reptilian brian [16] have been reported. The fatty acid composition of structural lipids is believed to be one of the factors that controls membrane physical properties and an inverse relationship between the content of unsaturated fatty acids and temperature has been reported for several poikilothermic organisms [17–19]. The structural membrane lipid composition, both the polar head group and non-polar fatty acyl chains of phospholipids, is also another important factor regulating the membrane physical properties during altered thermal environment [20–23]. Most of this information is based on studies from cold and temperate climates and there is a paucity of constructive information on tropical and sub-tropical poikilotherms.

In *Clarias batrachus*, an air-breathing teleost of equatorial and tropical climates, activity is reduced during summer aestivation like that of winter hibernation [24]. The Indian air-breathing teleosts including *C. batrachus* survive under the mud during long periods of summer drought. Hence, it would be more interesting to know about the mechanism of warm adaptation with regards to membrane physical state and the composition of the membrane lipid bilayer. The objectives of the present paper are first to understand the membrane lipid composition and the micro-viscosity during summer (when the ambient water temperature rises above 37°C with partial or semi-partial summer drought) and winter (when the ambient water temperature is about 16°C) and then to explore the possible regulation of these parameters during thermal adaptation of brain.

2. Materials and methods

2.1. Animals

The live fishes, *Clarias batrachus*, 100–150 g size, were collected from the local supplier during summer (ambient temperature 38° ± 3°C) and winter (ambient temperature 16° ± 3°C). They were acclimatized for 5 to 6 days to laboratory conditions at respective summer and winter temperature before scarificing them. Besides, the fishes were also collected during autumn (ambient temperature 28° ± 3°C).

2.2. Fractionation of brain membranes

Different brain membranes (viz., myelin, synaptosomes and mitochondria) were isolated and purified as described earlier [12]. The purity of different membrane fractions was determined by assaying of specific marker enzymes such as acetylcholinesterase (70% activity was recovered in synapotsomes) and succinic dehydrogenase (65% activity was recovered in mitochondria) or the quantity of cerebocides (85% of total cerebocide was recovered in myelin) as per our earlier experience [12]. For each set of membrane preparation the brains from 15 fishes were pooled. The membranes were stored at −80°C for measuring the membrane microviscosity. Otherwise, they were stored at −20°C prior to extraction of membrane lipid on the following day.

2.3. Fluorescence polarization

The membrane fluidity was measured by measuring the fluorescence polarization of 1,6-diphenyl 1,3,5-hexatriene as described previously [7,12]. The different membrane fractions, about 200 μg of protein [25], were incubated in 3 ml of 0.05 M Tris-HCl buffer (pH 7.2) containing 10 μl of diphenyl hexatriene (1.0 mM in tetrahydrofurane) for 30 min at room temperature (25°C) and measurements were taken from 5° to 45°C at intervals of 2.5°C.

2.4. Qualitative and quantitative analysis of membrane lipids

The total lipid from each membrane fraction, already suspended in Tris-HCl buffer, was vigorously

shaken for 5–6 h at room temperature with excess of (1:10, v/v ratio) solvent chloroform and methanol (2:1, v/v) and 1 mM BHT. The different lipid classes (neutral, glyco and polar lipids) were separated and isolated on hand-made silica gel-G (0.2 mm thick) TLC plates using hexane and then acetone as solvent. The glycolipid fraction was not further analyzed. The neutral lipid fraction as well as polar lipid fraction were further separated on silica gel TLC plates using either hexane:diethylether:acetic acid (85:15:1.5, v/v) or chloroform:methanol:water (65:25:5, v/v), respectively, as the solvent system. The spots were exposed to iodine vapour and identified using authentic (M/s, Sigma Chemical Co.) standards. Each spot was quantified spectrophotometrically by routine analytical methods [26]. The triacylglycerol and diacylglycerol by chromatropic acid reagent, total cholesterol by ferric chloride reagent and phospholipid by ammonium molybdate reagent were estimated and expressed as per unit of total protein. Some of the phospholipid fractions were transmethylated to analyze the fatty acid profiles [12].

Besides, the phosphatidyl-choline and -ethanolamine fractions from the total brain phopholipid, collected during summer and winter were digested with phospholipase A_2, EC 3.1.1.4, (Snake Venom, *Naja naja*, M/s, Sigma Chemical Co.) in order to analyse the fatty acid profiles of *Sn*-1 and *Sn*-2 positions. [6].

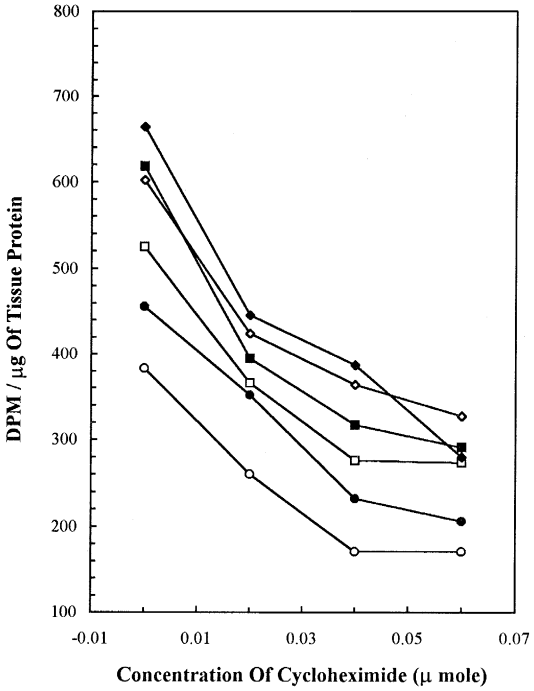

Fig. 1. Effect of cycloheximide on the incorporation of [1-^{14}C]glycine into brain tissue protein of *Clarias batrachus* at 37° and 16°C. The brain slices (500 mg) were incubated with 2 μCi of radiolabelled glycine and with different concentrations (0.02, 0.04, 0.06 μmol) of cycloheximide up to 3 h, in order to check the inhibition of protein synthesis. There is no statistically significant difference in the level of inhibition measured at 37°C (solid symbols) and 16°C (open symbols) irrespective of the time (1 h, circle symbols; 2 h, square symbols; and 3 h, rhombus symbols) of incubation. Each value is the mean of four different sets of experiment.

2.5. Tissue labelling experiment

Activation of phospholipase, acyltransferase, fatty acid synthetase, desaturase during thermal adaptation to regulate the membrane lipid bilayer is reported for several cases [6,23,27]. In order to check for cellular phospholipase activation, brain slices (500 mg, 1 mm thickness from 6–7 fishes collected during autumn) were incubated in 10 ml of Kreb's Ringer phosphate buffer (pH 7.5), containing sodium salt of [1-^{14}C]palmitic acid, 2 μCi, and or [1-^{3}H]arachidonic acid, 2 μCi, at room temperature for 1 h. The slices were washed thoroughly with 5 mM of sodium salt of cold fatty acid to remove unreacted surface bound fatty acids. The slices were further incubated in Kreb's ringer phosphate buffer for another 3 h at 16°, 37°C and room temperature (25°C). A steady-state incorporation of [1-^{14}C]sodium acetate in the total lipid and [1-^{14}C]glycine in the total protein of brain tissue slices in this buffer medium was noticed 8 h (at 16°C) or 4 h (at 37°C). To prevent the synthesis of any new protein during the thermal treatment of the slices, in a separate set of experiments we added 0.04 μmol of cycloheximide before incubating the slices at different temperatures. In earlier studies, we observed that at this concentration of cycloheximide the incorporation of [1-^{14}C]glycine into the brain tissue protein was blocked by about 50% irrespective of temperature and time of the incubation (Fig. 1). After the incubation, the total lipids from the slices were further separated into total phospholipid and total free fatty acid fractions on hand-made silica gel-G plates in order to determine the incorporation of labelled

fatty acids. Counts were corrected for quenching and counting efficiency.

2.6. Materials

The lipid standards, phospholipase A_2, fatty acids, DPH were from M/s, Sigma Chemical Co. The radiolabelled fatty acid viz. palmitic and arachidonic acids (spec. act. 40–60 μCi/μmol) were from M/s, Amersham. The solvents used were laboratory distilled and all other chemicals used were in analytical grade.

3. Results

3.1. Fluorescence polarization value

There is an inverse relationship between polarization value and membrane fluidity. From Fig. 2 it is clear that the mitochondrial membranes are more fluid in comparison to other membranes, irrespective of season. The order of membrane physical state (fluidity) is as follows: mitochondrial membrane > synaptosomal membrane > myelin membrane. Myelin and mitochondrial membranes from summer adapted fish show about 10–35% higher ($P < 0.01$) polarization values, between 5° and 45°C, than those of the

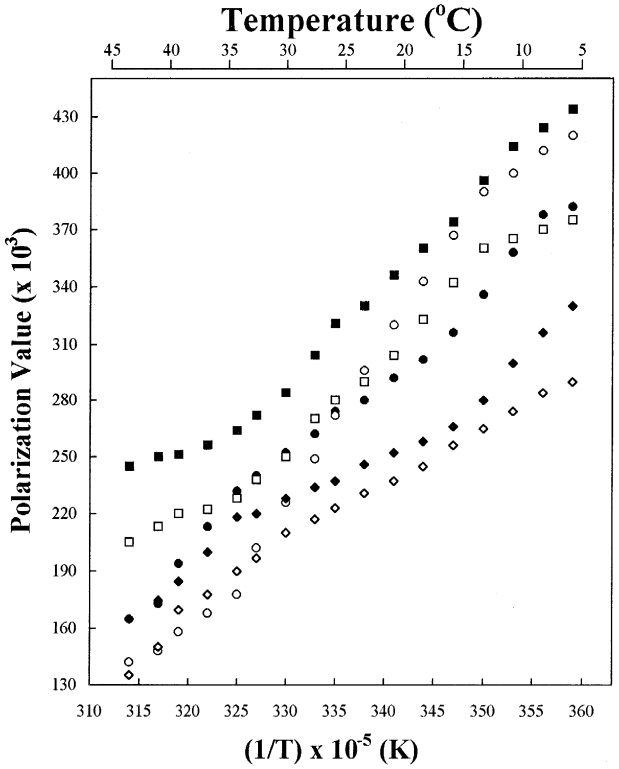

Fig. 2. The Van 't Hoff plots of DPH polarization value of different brain membranes, viz., myelin (square marks), synaptosomes (circle marks) and mitochondria (rhombus marks) of summer (solid marks) and winter (open marks) adapted *Clarias batrachus*. Each value is the mean of four different sets of membrane preparations.

Table 1
Seasonal variation in brain membrane lipid components of *Clarias batrachus*

	Myelin		Synaptosomes		Mitochondria	
	Winter	Summer	Winter	Summer	Winter	Summer
Phospholipid	1357 ± 30.27	912.64 ± 22.35	828.18 ± 20.18	883.00 ± 243.37	368.71 ± 18.25	232.67 ± 13.37
Diacylglycerol	568 ± 0.46	2.98 ± 0.38	5.33 ± 0.28	3.94 ± 0.17	1.24 ± 0.13	0.78 ± 0.12
Cholesterol	11.36 ± 0.67	9.45 ± 0.48	13.7 ± 0.52	11.58 ± 0.45	3.24 ± 0.16	2.29 ± 0.13
Triacylglycerol	3.46 ± 0.27	1.06 ± 0.18	2.54 ± 0.18	1.72 ± 0.15	0.99 ± 0.23	0.29 ± 0.08
Cholesterol/phospholipid ratio	8.37×10^{-3}	10.35×10^{-3}	16.54×10^{-3}	13.11×10^{-3}	8.78×10^{-3}	9.84×10^{-3}
Cardiolipin	14.09 ± 1.05	19.45 ± 1.18	17.16 ± 1.89	18.24 ± 2.24	14.04 ± 1.43	18.09 ± 1.95
Phosphatidic acid	10.06 ± 0.95	10.51 ± 0.88	12.06 ± 0.98	7.66 ± 0.69	9.25 ± 0.72	7.88 ± 0.67
Phosphatidylethanolamine	16.35 ± 1.17	17.50 ± 1.08	14.22 ± 1.03	16.28 ± 1.13	15.57 ± 1.21	15.88 ± 1.13
Phosphatidylserine + inositol	19.77 ± 1.38	6.92 ± 0.66	12.40 ± 1.01	10.76 ± 0.88	21.11 ± 1.89	13.72 ± 1.12
Phosphatidylchline	15.89 ± 1.76	28.48 ± 2.16	15.08 ± 1.23	28.96 ± 2.16	15.42 ± 1.15	28.62 ± 2.28
Sphingomyelin	12.58 ± 0.78	10.12 ± 0.64	12.80 ± 0.80	10.02 ± 0.46	9.38 ± 0.68	5.38 ± 0.28
Lysophosphatidylchloine	11.26 ± 1.03	7.01 ± 0.58	14.28 ± 1.12	10.03 ± 0.54	15.23 ± 1.37	10.42 ± 0.72

The different lipid components are expressed as nmol/μg of membrane protein. The phospholipid fractions are expressed as percent yield composition. All the data are the mean value of 4 different sets of membrane preparations with their standard error.

Table 2
Seasonal variation in the fatty acid profiles of phosphatidylcholine, phosphatidylethanolamine and sphingomyelin in the different brain membranes of *Clarias*

Fatty acids	Phosphatidylcholine						Phosphatidylethanolamine						Sphingomyelin					
	Myelin		Synaptosome		Mitochondria		Myelin		Synaptosome		Mitochondria		Myelin		Synaptosome		Mitochondria	
	Winter	Summer	Winter	Summer	Winter	Summmer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter	Summer
12:0	1.01	1.32	1.27	1.31	0.27	0.51	2.33	5.39	4.70	7.03	2.89	2.57	1.43	2.50	1.23	4.20	1.81	3.17
14:0	8.68	9.56	5.84	11.42	6.17	10.50	5.01	8.58	3.42	6.27	11.78	17.27	6.16	10.68	6.28	4.69	10.26	18.90
16:0	21.69	31.76	10.15	25.54	4.15	15.94	25.0	22.22	27.10	30.15	15.08	16.14	18.74	15.90	7.85	11.91	8.28	16.38
16:1	9.88	1.5	11.28	10.78	6.35	8.99	1.66	3.68	0.61	1.72	3.09	2.78	10.06	8.91	3.27	2.89	2.12	1.51
18:0	8.23	16.8	8.27	10.06	15.16	25.62	11.70	18.18	10.13	15.82	8.06	14.37	8.42	20.95	18.24	33.92	8.17	13.42
18:1	19.21	15.62	25.27	17.26	27.17	13.34	14.00	10.37	22.9	15.76	20.07	16.36	28.73	13.85	20.87	12.63	25.67	17.09
18:2,n6	3.54	3.60	11.27	10.09	6.75	4.25	4.33	3.52	10.45	8.26	8.47	6.17	4.13	4.72	10.27	8.30	8.72	5.51
18:3,n3	3.10	0.90	2.15	0.88	3.16	2.86	1.67	0.87	2.36	1.82	5.16	3.82	1.15	0.91	4.28	2.82	2.22	1.79
20:1	0.98	1.30	1.28	0.74	1.36	2.16	0.67	0.8	1.13	1.05	2.65	2.80	2.12	0.68	2.10	1.10	2.24	1.27
20:2,n6	0.81	0.78	2.36	0.32	3.18	2.09	2.67	1.86	0.17	0.10	1.24	0.95	0.36	0.68	4.05	6.49	4.72	3.89
20:4,n6	2.02	2.80	7.27	4.06	6.38	5.16	3.33	3.12	2.86	2.64	3.92	2.16	4.67	3.41	5.71	3.61	4.03	3.51
20:5,n3	8.51	5.50	3.45	0.19	6.04	4.12	2.67	1.75	3.15	1.96	1.44	0.76	1.84	1.14	3.27	1.44	3.20	1.58
22:2,n6	4.71	2.22	2.17	0.42	1.37	1.20	6.67	5.12	0.15	0.32	2.27	1.75	1.12	1.82	2.87	2.15	2.15	2.41
22:4,n6	3.19	1.80	1.36	1.87	3.15	2.19	5.00	4.33	1.39	1.03	1.65	1.14	0.99	2.04	1.82	1.62	1.32	0.86
22:5,n3	2.12	0.71	1.36	0.53	2.18	1.19	5.00	3.16	2.18	1.12	2.48	1.34	3.18	3.8	1.32	0.52	1.84	1.19
22:6,n3	2.25	2.85	5.25	3.72	7.16	2.88	8.33	5.05	8.04	5.95	9.71	5.84	6.36	8.01	5.67	1.66	12.27	7.52

Each value is the representative of mean value of 4 different sets of membrane preparations.

winter adapted fish. There is no difference in the polarization value of synaptosomal membrane of summer and winter adapted fish measured at 25°C. However, the same membrane from summer adapted fish displays 15–20% higher ($P < 0.01$) polarization values between 25° to 45°C and 15–20% lesser ($P < 0.02$) polarization values between 5° and 25°C compared to the same membrane of winter adapted fish.

3.2. Characterization of membrane lipid bilayer

From Table 1 it is evident that the membrane lipid bilayers are composed of phospholipid (232–1357 nmol), cholesterol (2.0–14.0 nmol), diacylglycerol (0.7–6.0 nmol) and triacylglycerol (0.2–3.5 nmol) per μg of membrane protein. There is a significant reduction ($P < 0.005$–0.05) in the concentration of all these lipid constituents, relative to protein in summer adapted fish in comparison to winter adapted fish except in phospholipids (per unit of protein) of the synaptosomal membrane fraction and in cholesterol (per unit of protein) of the myelin fraction. Phosphatidylcholine is the most abundant phospholipid in all membranes followed by phosphatidylethanolamine or cardiolipin. We failed to distinctly separate phosphatidyl-serine and -inositol under our chromatographic conditions. Phosphatidylcholine is increased by almost 2-fold ($P < 0.01$) without any change in the level of phosphatidylethanolamine in summer in all membranes giving rise to a higher choline/ethanolamine ratio in summer adapted fish. In summer adapted fish there is an approximately 40% increase ($P < 0.025$ in the concentration of cardiolipin in the myelin membrane fraction at the expense of phosphatydyl-serine + -inositol, and 35% decrease ($P < 0.01$) in the level of phosphatic acid in the synaptosomal membrane fraction. Moreover, there is a decrease in the proportion of phosphatidyl-serine + -inositol of 65% in the myelin ($P < 0.01$) and about 35% in the mitochondrial membrane, ($P < 0.025$) and about 20–40% decrease ($P < 0.01$–0.05) in the concentration of sphingomyelin and lysophosphatidylcholine in all the membranes investigated (Table 1).

In all membranes, the levels of 16:0, 18:1, 18:3, and 20:5 in phosphatidylcholine show a seasonal variation ($P < 0.01$–0.05). Statistically significant changes ($P < 0.01$–0.05) in the level of 14:0 (in synaptosomes and mitochondria); 18:0 (in myelin and mitochondria); 22:2 and 22:5 (in myelin and synaptosomes); 12:0, 18:2, 22:6 (in mitochondria); 22:4 (in myelin); 20:1, 20:2, 20:4 (in synaptosomes) between summer and winter adapted fishes are observed (Table 2). In phosphatidylethanolamine, significant changes ($P < 0.01$–0.05) in the levels of 14:0, 18:0, 18:1, 22:6 (in all membranes); 12:0, 16:1, 22:5 (in myelin and synaptosomes); 18:2 (in synaptosomes and mitochondria); 18:3 (in myelin); 20:5 (in mitochondria) are evident between winter and summer (Table 2). The sphingomyelin fraction is characterized by significant changes ($P < 0.01$–0.05) in the concentration of 16:0, 18:0, 18:1 (in all membranes); 12:0, 18:2, 20:5, 22:6 (in synaptosomes and mitochondria); 14:0 (in myelin and mitochondria); 20:1 (in myelin and synaptosomes); 18:3, 20:2, 22:5 (in synaptosomes) during seasonal variation (Table 2).

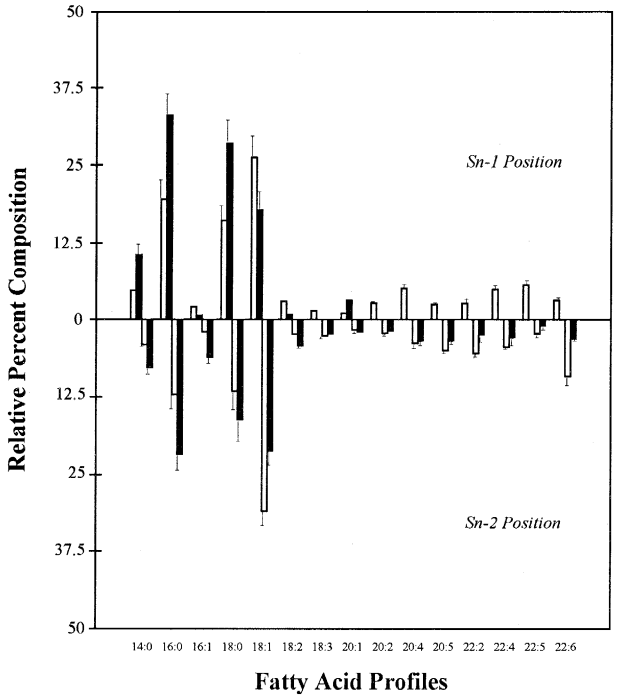

Fig. 3. Seasonal variation of the fatty acid profiles in *Sn*-1 and *Sn*-2 position in brain phosphatidylethanolamines of *Clarias batrachus*. The open bars represent the values (mean ± S.E. of four samples) from the winter adapted fish and the solid bars represent the values from the summer adapted fish.

3.3. Positional distribution of fatty acids in phosphatidyl-ethanolamines and -cholines

In the *Sn*-1 position of phosphatidyl-ethanolamine and -choline, saturated (14:0, 16:0, 18:0) and monoenoic (16:1, 18:1, 20:1) fatty acids are dominating (particularly in summer). Polyunsaturated fatty acids appear in this position only during the winter season. In *Sn*-2 position almost all fatty acids are represented (Figs. 3 and 4). In *Sn*-1 position of phosphatidylethanolamine an elevated level ($P < 0.01$) of 14:0, 16:0, 18:0, 20:1 is observed in summer. Adaptation to winter brought about an accumulation of 18:1, 16:1, 18:2 ($P < 0.025$) and other polyunsaturated fatty acids in the *Sn*-1 position of phosphatidylethanolamine. In summer adapted fish, *Sn*-2 position of phosphatidylethanolamine is characterized by significant ($P < 0.25$) higher level of 14:0, 16:0, 16:1, 18:2 and accompanied by low level ($P < 0.05$) of 18:1, 22:2, 22:4, 22:6 (Fig. 3). The *Sn*-1 position of phosphatidylcholine of summer fish is characterized by the significant increase ($P < 0.01$) in 14:0, 16:0, 18:0 and significant decrease ($P < 0.01$) in 18:1 and the absence of polyunsaturated fatty acids. The *Sn*-2 position of phosphatidylcholine from winter adapted fish is marked by decreased level ($P < 0.025$) of in 14:0, 16:0, 18:0, 18:2, 20:2, in contrast there is a significant increase ($P < 0.05$) in 18:3, 20:5, 22:2, 22:6 fatty acids (Fig. 4).

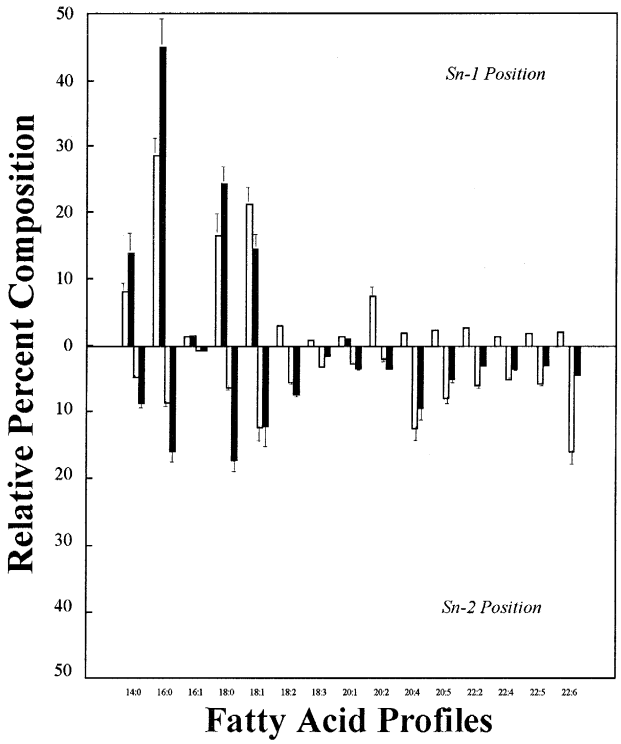

Fig. 4. Seasonal variation of the fatty acid profiles in *Sn*-1 and *Sn*-2 position in brain phosphatidylcholines of *Clarias batrachus*. For the rest of the legends refer to Fig. 3.

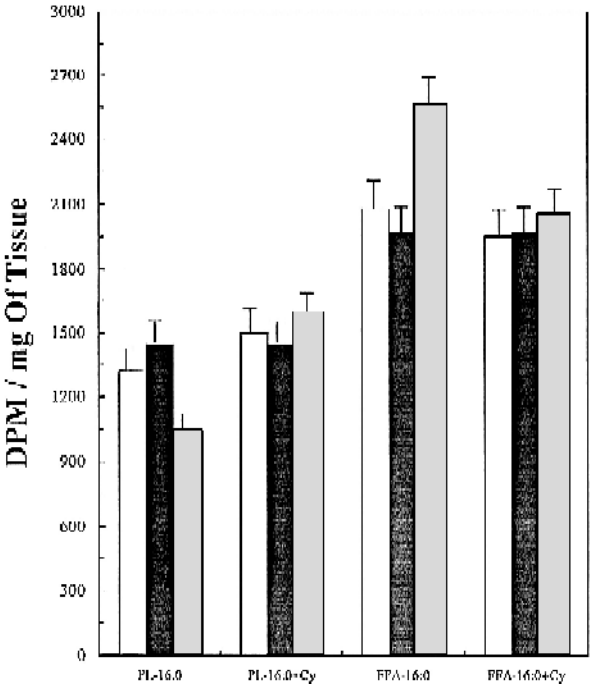

Fig. 5. The effect of temperature on the cellular phospholipase activity in the brain of *Clarias batrachus*. A tissue labelling experiment with [1-^{14}C]palmitic acid (see Section 2) in the presence or absence of cycloheximide. The experiment has been conducted at 16° (open bars), and at 37°C (shaded bars) over the control, i.e., at 25°C (closed bars). The plotted values are the mean of four sets of experiment ± S.E.

3.4. Labelling experiment with 1-^{14}C 16:0 and 1-^{3}H 20:4

While the counts (DPM/mg of tissue wt.) in the total phospholipid fraction decreased at 37°C irrespective of the label, there is a significant decrease ($P < 0.01$) in the counts at 16°C with 1-^{3}H 20:4 (Figs. 5 and 6). No significant change in the incorporation of 1-^{14}C 16:0 is detected in the free fatty acid fraction at 16°C, but the counts are significantly increased ($P < 0.025$) with 1-^{3}H 20:4 labelling at

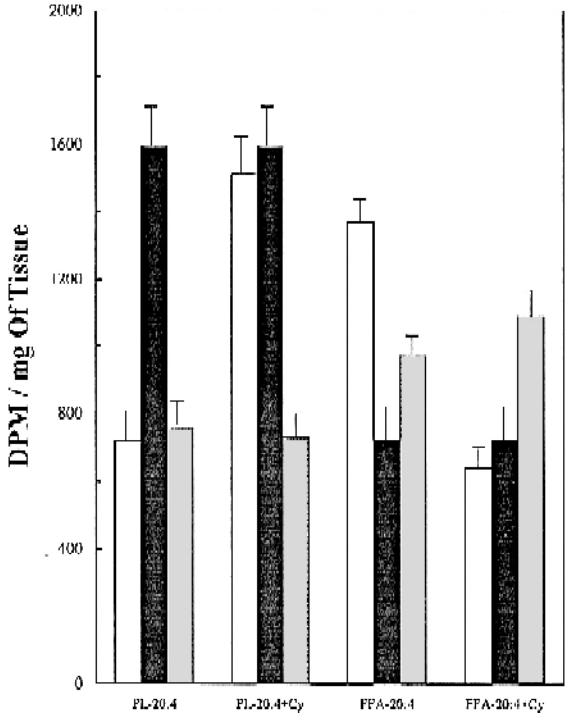

Fig. 6. The effect of temperature on the cellular phospholipase activity in the brain of *Clarias batrachus*. A tissue labelling experiment with [1-^{14}C]arachidonic acid in the presence or absence of cycloheximide. For rest of the legends refer to Fig. 5.

both incubation temperatures (low and high). When 0.04 μmol of cycloheximide was used in the second incubation medium maintained at 16° and 37°C to prevent the synthesis of any new protein, a significant decrease ($P < 0.01$) in the counts of phospholipid fraction is observed only at 37°C with 1-^{3}H 20:4 and at the same time the counts in free fatty acid fraction increase significantly ($P < 0.025$) at this temperature and this particular labelling experiment (Figs. 5 and 6).

4. Discussion

DPH fluorescence polarization has been extensively used to estimate membrane microviscosity [7,12,28]. Polarization values indicate the degree of hinderance to the rotation of the DPH probe in the matrix of membrane and thus it is an index of membrane rigidity or fluidity. It is quite difficult to define precisely the membrane fluidity or microviscosity mainly due to the complexity of the membrane lipid bilayer structure and its association with several protein and sugar moieties. Whatever values are obtained from DPH fluorescence polarization, it is the mixture of motions, ranging from isomerization within hydrocarbon chain, to wobbling and rotational motions of an entire lipid molecule and the lateral diffusion within the plane of membrane matrix [29]. However, in its broadest sense in which the term is commonly used, membrane 'fluidity' encompasses a combination of both oriental as well as dynamic properties of the constituents of membrane lipid bilayer.

It is clear from Table 1 that, although the phospholipid molecules are the main constituents of membrane lipid bilayer, other lipid molecules, like cholesterol and to some extent the triacylglycerol and diacylglycerol, may also contribute in maintaining the membrane physical state. The presence of triacylglycerols and diacylglycerols has not been reported so far in these types of membranes. However, the diacylglycerol is known as an intermediate product of biosynthesis of phospholipid molecules and it is also the intermediate product during the inter-conversion of several phospholipid molecules [30]. The first step in the synthesis of amino phospholipids is the hydrolysis of phosphatidic acid (a parent phospholipid molecule) to diacylglycerol. Phosphatidate phosphohydrolase, a membrane-bound enzyme, exhibits nearly equal specific activities in all the subcellular fractions of rat liver [31]. Furthermore, diacylglcerol is an important messenger during signal transduction [32].

The Van 't Hoff plots of apparent fluidity (DPH polarization value) for mitochondrial and myelin membrane fractions of *Clarias batrachus* (Fig. 2) indicate a translational shift to the left of the polarization curve in summer adapted fish. This ensures the maintenance of membrane microviscosity at the high environmental temperature. In other words, the tropical air breathing fish, *Clarias*, is capable of homeoviscous adaptation, 'partial compensation' [33] of its brain mitochondrial and myelin membrane as reported for other teleosts [10–12,34]. It is intriguing to note that the pattern of seasonal adaptation in brain synaptosomal membrane is very different from that of any other brain membrane (Fig. 2). It appears that the synaptosomal membrane fraction of this tropical

air breathing teleost is more capable of adapting to a higher (25– 45°C) than a lower (5–25°C) thermal regime. This indicates an 'inverse compensation' [33] of microviscosity during the winter season. It is noteworthy in this connection that this fish cannot survive at temperatures below 14°C although it is capable of adapting to a higher temperature such as 38°C.

The observed decrease in the ratio of individual lipid components (per unit of protein) in summer adapted *Clarias batrachus* (Table 1) might be due to the utilization of lipid molecules to meet the demand for energy to maintaining the normal physiological processes. The cold adaptation is characterized by 'bio-synthetically directed metabolic reorganization' [1] and the warm adaptation is marked by a catabolic reorganization of the biomolecules. Although the apparent concentration of cholesterol (in terms of protein) is higher in winter (Table 1) in all membranes, the ratio of cholesterol to phospholipid shows an inverse relation to the temperature in myelin and mitochondrial fractions. The cholesterol is known as 'packing' material in the phospholipid matrix and contributes to the ordering of the membranes. In some membranes, like muscle mitochondrial and hepatic microsomal membranes of carp, the cholesterol to phospho-lipid ratio increases at higher growth temperature [35,36], but in some other membranes, like the brain synaptic and myelin fraction of gold fish, this ratio does not alter at higher temperature [10,37].

Cold adaptation of membranes is generally associated with an increase in the relative concentration of phosphatidyl-ethanolamine and less frequently, with a reduction in the level of phosphatidyl-choline [38]. However, in the brain membranes of Indian (tropical) air-breathing teleost, warm adaptation resulted in an increase in the relative concentration of phosphatidyl-choline with no change in the concentration of phosphatidyl-ethanolamine. Reduction in the levels of sphingomyelin, lysophosphatidylcholine, phosphatidyl-serine + -inositol were also observed (Table 1). Warm adaptation is generally characterized by the increase in the relative proportion of 'cylindrical'-shaped lipids, having large hydrophobic surface volumes. In contrast, increase in the concentration of 'inverted cone' or 'wedge-shaped' lipid molecules with small hydrophobic surface volume are preferred during cold adaptation. The geometry of lipid components determines the stability and phase composition of the membranes which in turn helps in understanding and interpreting the consequences of temperature-induced alteration in membrane lipid composition [39,40]. Conically-shaped molecules easily form non-bilayer (H_{II}) phases and it has been proposed that for normal membrane function a precise balance between bilayer and non-bilayer forming lipids is required [41]. The bilayer-non-bilayer transition temperature of phospholipid molecules containing monoenoic fatty acids in *Sn*-1 position and a polyunsaturated fatty acid in *Sn*-2 position are lower than those containing saturated fatty acids in position *Sn*-1 [42,43].

Seasonal variation of environmental temperature triggers the processes aimed at restructuring the existing phopholipid molecules of the membrane. Rapid retailoring of the existing phopholipid molecules during thermal adaptation has been reported [6,44,45]. Activation of phospholipase(s) and acyltransferase are one of the pre-requisites for such structural reorganization of the phospholipids. Activation of phospholipase A_2 in liver microsomes of trout [27] or phospholipase A_1 in carp liver [6] during cold adaptation has been reported. In the present study we have noticed that phospholipase A_1 is activated at higher growth temperature and phospholipase A_2 is at lower growth temperature in the brain of *Clarias* (Figs. 5 and 6). However, it is interesting to note that the liberation of [1-^{3}H]arachidonic acid at higher growth temperature is not blocked by the cycloheximide (Fig. 6).

The physical parameter, like surface area and thermotropic phase transition temperatures, of phospholipid molecules containing polyunsaturated fatty acids are rather close [46,47]. Similar or greater changes in fatty acid composition of synaptosomal membranes of gold fish [10], microsomal membrane of green sunfish [11] and total phospholipids in liver and brain of *Channa punctatus* [7,12] as well as in the present study resulted in only a partial compensation of membrane fluidity. It is probable that alteration of gross fatty acid compositions is only a reflection of changes taking place at the structural level of membrane and that restructuring of the existing phospholipids is more important in this context. Figs. 2 and 3 suggest that some reorganization of molecular archi-

tecture and molecular species composition of phospholipids might have occurred during seasonal adaptation. 1-monoenoic, 2-polyenoic molecular species are elevated as a result of cold adaptation in trout liver microsomes [45] in the liver and brain of fish [6,13,48], shrimps [17].

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References

[1] Hochachka, P.W. and Somero, G.N. (1984) Biochemical Adaptation, Princeton University Press.
[2] Sinensky, H. (1974). Proc. Natl. Acad. Sci. USA. 71, 522–525.
[3] Singer, S.J. and Nicholson, G.L. (1972) Science 175, 720–731.
[4] Hazel, J.R. and Williams, E.E. (1990) Prog. Lipid Res. 29, 167–227.
[5] Farkas, T. and Csengeri, I. (1976) Lipids 11, 401–407.
[6] Farkas, T. and Roy, R. (1989) Comp. Biochem. Physiol. 93B, 217–222.
[7] Dutta, H., Das, A., Das, A.B. and Farkas, T. (1985). Comp. Biochem. Physiol. 81B, 341–347.
[8] Dey, I., Buda, C., Wilk, T., Halver, J.E. and Farkas, T. (1993) Proc. Natl. Acad. Sci. USA 90, 7498–7502.
[9] Abruzzini, A.F., Ingram, L.O. and Clem, L.W. (1982) Proc. Soc. Exp. Biol. Med. 169, 12–18.
[10] Cossins, A.R. (1977) Biochim. Biophys. Acta 470, 395–411.
[11] Cossins, A.R., Kent, J. and Prosser, C.L. (1980) Biochim. Biophys. Acta 599, 341–358.
[12] Roy, R., Ghosh, D. and Das, A.B. (1992) J. Therm. Biol. 17, 209–215.
[13] Buda, C., Dey, I., Balogh, N., Horvath, L.T., Maderspach, K., Juhasz, M., Yeo, Y.K. and Farkas, T. (1994) Proc. Natl. Acad. Sci. USA 91, 8234–8238.
[14] Di Costanzo, G., Duportail, G., Florentz, A. and Leray, C. (1983) Mol. Physiol. 4, 279–290.
[15] Lagerspitz, K.Y.Z. and Laine, A. (1984) Mol. Phys. 6, 211–220.
[16] Durairaj, G. and Vijaykumar, I. (1984) Biochim. Biophys. Acta 770, 7–14.
[17] Farkas, T., Nemecz, G. and Csengeri, I. (1984) Lipids 16, 418–422.
[18] Roy, R., Das, A.B. and Farkas, T. (1991) J. Therm. Biol. 16, 211–216.
[19] Chapelle, S. (1978) J. Exp. Zool. 204, 337–346.
[20] Hazel, J.R. (1983) J. Exp. Zool. 227, 167–176.
[21] Hazel, J.R, and Carpenter, R. (1985) J. Comp. Physiol. 155, 597–602
[22] Sellener, P.A. and Hazel, J.R. (1982) J. Exp. Zool. 221, 139–168.
[23] Schunke, M. and Wodtke, E. (1983) Biochim. Biophys. Acta 734, 71–75.
[24] Hora, S.L. and Pillay, T.V.R. (1962) FAO Fish. Biol. Tech. Paper 39, 112–158.
[25] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randal, R.J. (1951) J. Biol. Chem. 193, 265–275.
[26] Kates, M. (1986) Techniques of Lipidology – Isolation, Analysis and Identification of Lipids, Elesevier, New York.
[27] Neas, N.P. and Hazel, J.R. (1985) J. Exp. Zool. 233, 51–60.
[28] Shinitzky, M. and Barenholz, Y. (1978) Biochim. Biophys. Acta 515, 367–394.
[29] Cossins, A.R. and Lee, J.A.C. (1985) in Circulation, Respiration and Metabolism: Current Comparative Approaches (Gilles R. ed.), pp. 543–552, Springer, Berlin.
[30] Longmuir, K.J. (1987) in Current Topics in Membrane and Transport (Bronner, F. ed.), Vol. 29, pp. 129–174, Academic press, New York.
[31] Jelsema, C.L. and Morre, D.J. (1978) J. Biol. Chem. 253, 960–971.
[32] Edwards, S.W. (1995) Trends in Biochem. Sci. 20, 362–367.
[33] Precht, H., Christophersen, J. and Larcher, W. (1973) Temperature and Life, pp. 293–354, Springer, New York.
[34] Cossins, A.R. and Prosser, C.L. (1982) Biochim. Biophys. Acta 687, 303–309.
[35] Wodtke, E. (1978) Biochim. Biophys. Acta 529, 280–291.
[36] Wodtke, E. (1983) J. Therm. Biol. 8, 416–420.
[37] Selvinonchick, D.P. and Roots, B.I. (1976) J. Therm. Biol. 1, 131–135.
[38] Hazel, J.R. (1988) in Physiological Regulation of Membrane Fluidity (Aloia, R.C., Curtain, C.C. and Gordon, L.M., eds.), pp. 149–188, Alan R. Liss, New York.
[39] Wieslander, E., Christiansson, A., Johansson, L.B.A., Rilfors, L. and Khan A. (1981) FEBS Lett. 124, 273–278.
[40] Rilfors, L., Lindblom, G., Wieslander, A. and Christiansson, A. (1984) Biomembrane 12, 205–245.
[41] Hazel, J.R. (1995) Ann. Rev. Physiol. 57, 19–42.
[42] Giorgione, J., Epand, R.M., Buda, C. and Farkas, T. (1995) Proc. Natl Acad. Sci. USA 92, 9767–9770.
[43] Farkas, T., Dey, I., Buda, C. and Halver, J.E. (1994) Biophys. Chem. 50, 47–155.
[44] Dickens, B.F. and Thompson, G.A. Jr. (1982) Biochemistry 21, 3604–3611.
[45] Hazel, J.R. and Zebra, E. (1986) J. Comp. Physiol. 156, 665–674.
[46] Coolbear, K.P., Berde, C.B. and Keough, K.M.W. (1983) Biochemistry 22, 1466–1473.
[47] Ghosh, D., Williams, M.A. and Tinoco, J. (1973) Biochim. Biophys. Acta 291, 351–362.
[48] Fodor, E., Jones, R.H., Buda, Cs, Katajka, K., Dey, I. and Farkas, T. (1995) Lipids 30, 1119–1126.