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Role of phospholipid molecular species in maintaining lipid membrane structure in response to temperature

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Abstract

The compositions and physical states of the liver phospholipids of fish and phospholipids of shrimps adapted to relatively constant but radically different temperatures were investigated. There were no measurable differences in their gross fatty acid compositions of phospholipids from the species obtained from identical temperature. Saturated-to-unsaturated fatty acid ratio did not show any convincing difference. Docosahexaenoic acid (22:6) did not seem to participate in the process of adaptation. Cold adaptation was coincidental with oleic acid (18:1) accumulation, preferentially in the phosphatidylethanolamine. In the experiment with rats, it has been found that the fish oil fed rats showed a similarity in their liver phospholipid fatty acid composition like fish liver phospholipids. Determination of molecular species composition of phosphatidylcholine and phosphatidylethanolamoine revealed a 4- to 5-fold and 10-fold increase in the level of 18:1/22:6 and 18:1/20:5 species respectively in favor of cold adaptation. Phospholipids from cold-adapted species showed a more fluid structure than that of warm-adapted species near the C-2 segment of the bilayer, but not in the deeper regions. Phospholipids from the rat livers did not show any change irrespective of diet. Phosphatidylcholines from rat liver, fish liver or shrimps did not show any difference among them in their fluidity irrespective of their environmental conditions or diet. An appropriate combination (75:25) of phosphatidylcholine from rat liver with phosphatidylethanolamines from cold adapted species showed a drastic fluidization at the C-2 segment, in comparison with their phosphatidylcholines. In the reverse combination it showed a rigidification at the same segment. Role of saturated-to-unsaturated fatty acid ratio in lipid membrane adaptation has been argued and the role of specific molecular species has been proposed.

Key words: Phospholipid; Molecular species; Fluidity

1. Introduction

Poikilotherms are often subjected to environmental stress. Temperature is one of the most important perturbing factors intervening in their life. Membranes in their cells are the first target of such kind of challenge. Physically and chemically membranes posses a highly heterogeneous structure. It has been generally agreed that the lipid component is responsible for adjusting membrane physical and chemical state to these conditions. It is expected that the response due to

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temperature change must be rapid, reversible and must not depend on the thermal history of the animal. These organisms use the diversity in lipid structures to fashion membranes to prevailing temperature in a way that they become more fluid in their cold-acclimated state and more rigid in their warm-acclimated state. The first evidence of such kind of regulation has been found in the late fifties by Farkas et al. [1,2]. It has been found that the melting point of the extracted lipids from some fresh-water crustaceans was always a few centigrade below the water/body temperature. In other words the lipids in these creatures were just in the melted state. Fifteen years later Sinensky [3] found that the thermotropic phase transition of phospholipid isolated from *E. coli* was always far below their growth temperature. He termed this phenomenon "homeoviscous adaptation". To our present knowledge there are several possibilities available for the poikilotherms to adjust membrane physical state to temperature. First, they can increase or decrease the number of double bonds in the existing constituent fatty acyl chains and thus by changing their saturated-to-unsaturated fatty acids ratios [4–6]. It is well known that insertion of a new double bond in the fatty acyl chain results in a decrease of the melting point of the fatty acids and partially the same is also true for the thermotropic phase transition of the phospholipids. The introduction of a *cis*-double bond into an acyl chain can affect both the molecular shape and physical properties of a phospholipid molecule. Because rotation is restricted about a carbon–carbon double bond, a bend of $\approx 30°$ is introduced into the acyl chain (Smith and Oldfield [7]). Furthermore, restructuring in the polar headgroups compositions, mainly in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) has also been shown to be responsible in thermal adaptation. It has been found that membranes of cold-adapted/acclimated poikilotherms routinely possess higher proportions of PE and less commonly, reduced proportions of PC than membranes at higher growth/acclimated temperatures [8]. Consequently PC/PE ratio tends to be positively correlated with growth/acclimated temperature [9–13].

If acyl chain and polar headgroup composition of a membrane changes significantly, it follows that the distribution of phospholipid molecular species must also change. However the converse of this statement is not true, for significant changes in molecular species composition may occur without an alteration in acyl chain or polar headgroup composition [14]. Thus it is possible for fatty acids to be "reshuffled" to form new molecular species of phospholipids without altering the fatty acid composition of the membrane. The potential significance of acyl chain reshuffling to the thermal adaptation of membranes was first illustrated in *Tetrahymena* [15] and later on in several other species, such as in the Sonoran desert teleost, *Agosia*, in response to diurnal warming [16], in rainbow trout undergoing acclimation from 20°C to 5°C, where proportions of monoenoic and disaturated molecular species declined significantly in renal plasma membrane [17]. Frequently, altered proportions of only a few molecular species accounts for the vast majority of the acclimatory response, suggesting that thermal adaptation of molecular species composition may involve a precise control of the balance between disaturated and diunsaturated phospholipid species [18]. Collectively these results suggest that the restructuring of phospholipid molecular species may be a common means of rapidly adjusting membrane composition to temperature challenge. Furthermore, since no input from the lipid biosynthetic apparatus is required, this mechanism may be metabolically inexpensive and particularly important when low temperature effectively stops the net phospholipid synthesis. The manipulation of preexisting lipids remains as the only alternative for the restoration of the function [19]. Moreover because of molecular species reorganization the physical properties of the membranes are also reorganized, thereby influencing the lipid membrane function in response to temperature [20].

All the above chemical changes in the lipid membranes are reflected as a change in the fluidity of the membrane, which directly gives us a view of physical organization of these molecules in the membrane. Thus any changes in lipid compositions are expected to be reflected by the

change of fluidity in the lipid membrane or in other words changes in the membrane fluidity also must indicate a change in the membrane lipid composition. There is a growing body of evidence that change in the unsaturation of fatty acids due to temperature adaptation or dietary manipulation is directly related with the changes in membrane fluidity.

The present article compares and justifies the relation between lipid membrane fluidity and saturation to unsaturation ratio in the fatty acids composition. An effort has been made to clarify lipid membrane fluidity through phospholipid molecular species reshuffling and restructuring.

2. Materials and method

Fish: Ophioidon elongatus (Ling cod) is from Seattle, USA (5–7°C) and *Pomadysys hasta* and *Nemipterus hexodon* are from the South-Chinese Sea (25–27°C). Livers from them were dissected immediately and were air-shipped in sealed vials in chloroform/methanol (2:1 v/v) in the presence 0.01% butylated hydroxytoluene (BHT) in dry ice.

Shrimps: Pandulus borealis is from north Norway (2°C) from −200 m and *Parapandulus sp.* is from Egypt, Mediterranean Sea at the coast of Alexandria (27–30°C). They were collected and transported in a similar way like fish livers. The fish and the shrimps were caught wild.

Experimental procedure with rats: In each case there were two sets of rats. In each set there were a minimum of six rats. One set was given normal rat food mixed with sunflower oil and another was fed with a mix of standard fish oil rich in long-chain poly-unsaturated fatty acids. They were treated for six weeks and after this time the rats were killed by stunt death and livers from them was dissected. They were minced and homogenized in chloroform/methanol with a blender type homogenizer.

Analytical techniques: Total lipids extracted from all the sources were extracted according to Folch et al. [21]. Neutral lipids and phospholipids were separated by silicic acid column chromatography by chloroform and methanol respectively. Phospholipid subclasses were separated on G60 silica gel plates (Merck) according to Fine and Sprecher [22]. For fatty acid analysis total phospholipids (TPL), phosphatidylcholines (PC) and phosphatidylethanolamines (PE) were transmethylated with methanol in the presence of 5% hydrochloric acid at 80°C for 2.5 h. Fatty acids were separated on 10% FFAP coated onto Supelcoport 80–100 mesh (Supelco) in a 2 m column (2 mm i.d.) with a Hitachi 263-80 gas chromatograph.

The molecular species compositions of PC and PE were determined according to Takamura et al. [23]. The dinitrobenzoyl derivatives of the diacylglycerols were separated by HPLC (Waters, model 440) on a Nucleosyl C_{18} column (5 μm particle size; 4 mm i.d. × 250 mm). Acetonitrile/2-propanol of HPLC grade (Carlo Erba, Milan, Italy) was used as mobile phase. Authentic standards (16:0/22:6, 18:0/22:6, 16:0/20:4, 18:0/20:4, 16:0/16:0, 18:0/18:0, 16:0/18:1, 18:1/18:1, 18:0/18:1) were used for identification moreover on relative elution times according to Bell and Dick [24].

Fluorescence anisotropy measurements: To ensure the consistency of fluorescence measurements, 250 μg of phospholipid was taken. This amount of phospholipid was taken in 500 μl of chloroform and 10 μl of 1 μm 2- and 12-(9-anthroyloxy) stearic acid (2- and 12-AS) and 16-(9-anthroyloxy) palmitic acid (16-AP) (Molecular Probes Inc. Eugene, Oregon) dissolved in tetrahydrofuran was added with thorough mixing. The solvent was removed under high vacuum and the vesicles were prepared as described by Sen et al. [25]. Measurements and calculations of steady-state anisotropy (R_{ss}) were performed according to Dey et al. [20]. The SE in separate determinations was always < 0.005.

3. Results and discussion

Table 1 and 2 show the major fatty acid composition of total phospholipid (TPL), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in shrimps (*Pandulus borealis* and *Parapandulus sp.*) and in fish (*Ling cod* and *Ne-*

Table 1
Major fatty acid composition of total phospholipids (TPL), phosphatidylcholine (PC) and phosphatidylethanolamines (PE) in shrimps from Norway and Egypt

Fatty acids	Norway [a]			Egypt [b]		
	TPL	PC	PE	TPL	PC	PE
14:0	2.07	2.70	1.27	1.35	1.25	0.36
16:0	14.07	22.22	10.05	8.95	14.85	6.98
16:1ω-7	3.67	5.47	9.35	4.40	4.50	1.73
18:0	4.47	1.82	1.75	8.04	13.97	6.10
18:1ω-9	16.02	25.51	15.0	8.59	14.51	5.96
18:2ω-6	1.53	1.69	0.74	0.49	0.42	trace
18:3ω-3	0.55	0.54	0.21	0.34	0.48	trace
18:4	0.37	0.25	0.16	trace	trace	trace
20:1	0.20	0.49	0.45	0.94	1.07	0.69
20:4ω-6	2.69	2.24	2.54	12.26	10.24	13.13
20:4ω-3	0.10	0.12	0.10	trace	0.47	0.23
20:5ω-3	33.44	22.95	31.16	26.0	17.51	28.86
22:1	0.12	0.15	0.49	2.04	8.0	4.61
22:4ω-6	trace	0.46	0.38	trace	trace	trace
22:5ω-6	trace	0.20	0.10	trace	trace	trace
22:5ω-3	0.91	0.44	1.41	0.66	trace	0.31
22:6ω-3	20.73	11.24	31.02	19.27	6.05	25.98
Σ sat	20.61			18.34		
Σ unsat	79.39			81.66		
sat/unsat	0.25			0.22		
Σ ω-6	4.25			12.75		
Σ ω-3	55.18			46.27		

[a] *Pandulus borealis* (2°C).
[b] *Parapandulus sp.* (25°C).

mipterus hexodon). Major dominating fatty acids are 16:0, 18:0, 18:1, 20:4, 20:5 and 22:6 in all the species irrespective of their geographical location and inhabiting temperature. In any of the cases saturated to unsaturated ratio did not show any notable change. Despite that, it has been found that the fluorescence anisotropy of different anthroyloxy fatty acids (2-AS, 12-AS and 16-AP) embedded in their phospholipid vesicles demonstrates, that the membranes from the cold-adapted species are more fluid than that of the warm-adapted species. This fluidization is rather obvious in the outer most segment of the bilayer, i.e. in the 2-AS segment (Figs. 1 and 2). Although it is found that cold-adaptation is consequential mainly with the increase of 18:1 and 20:5, whereas warm-adaptation is paralleled by the increase of 18:0 and 20:4 particularly in PE. Increased level of 20:5 in cold-adapted species might have of dietary origin: in cold water bodies the diatomaceae, rich in this fatty acid, predominate, while, 18:1 might be the result of its preferential formation. Docosahexaenoic acid remained unchanged in all the cases. From our previous study with fish liver [26], it has been found that during the cold adaptation more 18:1 is directed towards the sn-1 position of PE. However, increase in the proportion of polyunsaturated fatty acid is not an absolute requirement for an effective control of membrane physical state. First, some species do not increase the level of polyunsaturated fatty acids (PUFA) at all. For instance the level of these fatty acids is almost identical in marine and fresh water fishes evolutionary adapted to contrasting temperatures [20]. It has also been found that fish erythrocytes are able to change their membrane fluidity with temperature without changing their fatty acid composition or saturated-to-unsaturated ratio [19,27]. This approximates that saturated-to-unsaturated fatty acid ratio or elevation in the level of PUFA does not play a decisive role in lipid membrane adaptation to temperature.

Furthermore, an indication that the gross amount of PUFA does not take part in controlling membrane fluidity comes from the experiment with rats. In this experiment one group of rat was maintained on a control diet with sunflower oil rich in linoleic acid and the other group was maintained on a fish oil diet, rich in ω-3 PUFA. Eventually, fatty acid composition of livers from fish oil fed rats resembled that of fish liver fatty acid composition (Table 3). It is obvious from Table 3, that the fish oil fed rats contains almost three times higher amount of ω-3 polyunsaturated fatty acids including a drastic increase in 20:5 and 22:6 both in total phospholipid and in PE. It is expected that these phospholipid vesicles from fish oil fed rats should also show a fluidization due to higher amount of 20:5 and 22:6 and lower amount of 20:4 fatty acids. It should be taken into consideration that 18:1 did not show any change with diet. Fig. 3, shows the anisotropy measurements with different anthroyloxy fatty acids (2-AS, 12-AS and 16-AP) incorporated in the phospholipid vesicle of such phospholipids extracted from rat livers. Despite their

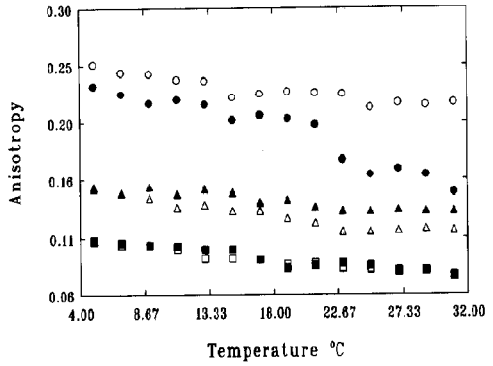

Fig. 1. Fluorescence anisotropy of 2- and 12-(9-anthroyloxy) stearic acid (2-AS and 12-AS) and 16-(9-anthroyloxy) palmitic acid (16-AP) in total phospholipids of cold-adapted fish *Ophioidon elongatus* and warm-adapted fish *N. hexodon*; (●) *Ophioidon elongatus*: 2-AS; (○) *N. hexodon*: 2-AS; (▲) *Ophioidon elongatus*: 12-AS; (△) *N. hexodon*: 12-AS; (■) *Ophioidon elongatus*: 16-AP and (□) *N. hexodon*: 16-AP.

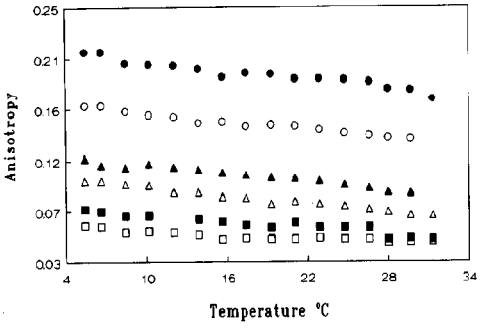

Fig. 2. Fluorescence anisotropy of 2- and 12-(9-anthroyloxy) stearic acid (2-AS and 12-AS) and 16-(9-anthroyloxy) palmitic acid (16-AP) in total phospholipids of shrimps from Norway and Egypt. (●), (▲) and (■), represents phospholipid from the shrimp of Egypt with 2-AS, 12-AS and 16-AP, respectively. (○), (△) and (□), represents phospholipids from the shrimps of Norway with 2-AS, 12-AS and 16-AP, respectively.

differences in PUFA composition and saturated-to-unsaturated fatty acid ratio these phospholipids did not show any difference in their fluidity. This signifies that gross change in the composition of PUFA is not playing the decisive role in controlling membrane ordering structure.

From our previous experience we know that molecular species composition might play a sig-

Table 2
Major fatty acid compositions of total phospholipids (TPL), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in livers of representative cold-adapted fish (*Ophioidon elongatus*) and warm-adapted fish (*Nemipterus hexodon*)

Fatty acid	Ophioidon elongatus			N. hexodon		
	TPL	PC	PE	TPL	PC	PE
14:0	1.186	1.541	5.551	0.540	1.372	0.751
16:0	14.759	18.027	14.959	14.290	24.113	16.353
16:1ω-7	2.504	1.375	1.405	0.547	1.025	0.281
18:0	7.845	6.437	7.083	14.324	6.263	18.346
18:1ω-9	14.310	9.694	11.726	7.410	6.413	2.041
18:2ω-6	0.704	0.397	0.113	0.401	1.259	trace
18:3ω-3	0.742	1.056	0.275	0.829	0.241	trace
18:4	2.926	1.073	0.086	0.315	0.159	trace
20:1	0.271	0.141	trace	0.251	0.171	trace
20:4ω-6	5.949	3.522	3.245	14.820	10.227	9.537
20:4ω-3	0.810	0.515	5.005	trace	0.122	0.096
20:5ω-3	24.268	22.233	13.980	3.700	4.148	1.643
22:1	trace	0.0809	0.651	trace	0.244	0.264
22:4ω-6	0.046	0.074	0.748	0.797	0.502	0.525
22:5ω-6	0.814	0.073	0.223	2.246	1.365	2.355
22:5ω-3	0.941	0.039	0.185	1.410	1.589	0.580
22:6ω-3	22.296	27.512	29.216	37.968	38.117	40.897
Σ sat	23.420			29.115		
Σ unsat	76.580			70.714		
sat/unsat	0.31			0.41		
Σ ω-3	49.057			43.907		
Σ ω-3	7.513			18.264		

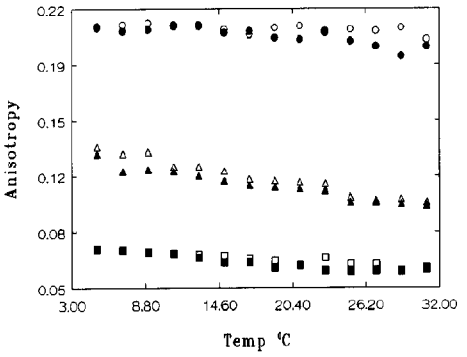

Fig. 3. Fluorescence anisotropy of 2- and 12-(9-anthroyloxy) stearic acid (2-AS and 12-AS) and 16-(9-anthroyloxy) palmitic acid (16-AP) in total phospholipids from rat livers treated with sunflower oil (control) and fish oil (treated) with (●), (▲) and (■) represents the phospholipids from the control rats with 2-AS, 12-AS and 16-AP, respectively. (○), (△) and (□) represents the phospholipids from the treated rats with 2-AS, 12-AS and 16-AP, respectively.

nificant role in controlling membrane structure to temperature. Tables 4 and 5 shows the molecular species composition of PC and PE from shrimps and fish respectively. It has been seen that in all the cases, in cold-adapted species there is 2-fold to 3-fold increase in the molecular species 18:1/22:6 and 18:1/20:5 mainly in PE but also evident in PC. On the other hand warm adaptation was paralleled by the increase of 18:0/22:6 and 18:0/20:4 in PE and PC. This result agrees with our previous finding with warm and cold adapted marine and fresh water fish [20]. Table 6 represents the molecular species composition of PC and PE from rat liver. It has been found that the fish oil fed rats showed a difference with sunflower oil fed rats like the difference between cold- and warm-adapted fish and shrimp respectively. PC and PE of fish oil fed rats are more rich in the molecular species 18:1/22:6 and 18:1/20:5 mainly in PE. It can be speculated from the above results that the molecular species composition is playing a decisive role in thermal adaptation.

The question remains, whether it is PC or PE molecular composition, that is affecting mainly the lipid membrane organization. It has been suggested from our previous study that some molecular species composition of PE (18:1/22:6

Table 3
Major fatty acid composition of rat liver total phospholipids (TPL), phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in relation to diet

Fatty acid	Sunflower oil			Fish oil		
	TPL	PC	PE	TPL	PC	PE
16:0	16.88 ± 1.40	15.24	16.70	12.04 ± 1.96	21.28	17.03
16:1	1.30 ± 0.56	0.77	0.27	1.65 ± 0.32	1.33	0.36
18:0	14.21 ± 0.91	11.27	22.57	13.50 ± 1.69	12.55	23.45
18:1ω-9	8.16 ± 0.83	5.24	5.08	8.20 ± 1.04	6.94	4.26
18:2ω-6	21.68 + 3.76	–	–	18.35 ± 0.56	–	–
18:3ω-3	0.48 ± 0.05	0.46	0.08	0.37 ± 0.09	–	0.11
20:3ω-6	0.73 ± 0.28	0.63	0.47	0.89 ± 0.12	1.01	0.47
20:4ω-6	28.00 ± 2.27	23.25	28.65	10.78 ± 1.94	14.17	11.0
20:5ω-3	0.47 ± 0.22	trace	0.28	9.72 ± 1.20	8.15	8.75
22:1ω-9	–	trace	trace	0.16 ± 0.07	1.58	0.11
22:4ω-6	0.74 ± 0.08	0.28	0.69	0.48 ± 0.07	trace	trace
22:5ω-6	0.10 ± 0.05	trace	0.15	0.23 ± 0.02	trace	0.08
22:5ω-3	1.15 ± 0.08	1.67	1.56	4.46 ± 0.69	3.97	4.10
22:6ω-3	8.23 + 1.13	5.28	11.03	16.20 ± 1.39	1.02	18.73
Σ sat	29.77			25.54		
Σ unsat	70.23			74.46		
sat/unsat	0.42			0.34		
Σ ω-6	51.25			19.95		
Σ ω-3	10.33			30.75		

PE and 18:1/20:5 PE) are more important than PC [20]. Considering the fact, that changes in PE molecular species composition is vital for lipid membrane regulation, vesicles were prepared from isolated PCs from all the samples including rat and their changes in fluidity were compared by labeling them with 2- and 12-AS and 16-AP. No significant changes in anisotropy could be detected among the PCs from any source. Fig. 4 represents such a measurement made on PCs from rat and cold-adapted species. In the next step phosphatidylcholines were cross combined with phosphatidylethanolamines from the other species, i.e. rat PC with fish PE rich in 1-monounsaturated/2-polyunsaturated species and fish PC with rat PE poor in these species. Fig. 4 shows an example of such a combination. It has been seen that the combined rat PC with fish PE vesicle shows a more fluid structure in the C-2 segment of the bilayer while rat PE had only a moderate effect on the fluidity of fish PC. At the deeper segment of the phospholipid membrane no changes could be observed (data not shown). The same results were also obtained when phosphatidylcholines from cold or warm adapted marine fish were cross combined with phosphatidylethanolamines of warm- and cold-adapted fish livers [20].

We propose that this spatial effect of 1-monounsaturated/2-polyunsaturated fatty acids can be explained by their specific molecular architectures. According to the recent calculations the long chain PUFAs presume a rather extended, so-called angle iron configuration [28], instead of hair-pin or helical configuration in the phospholipids. This explains, for instance the similarities in anisotropy values in the phospholipids vesicles from rat livers fed with sunflower oil and fish oil, though differences in their molecular species could be observed. Introduction of a *cis*-double bond into the sn-1 position of such phospholipids, for instance the phosphatidylethanolamines will increase the surface area of the molecule. Proba-

Table 4
Molecular species composition of di-acylphosphatidylethanolamines and phosphatidylcholines of shrimps from Norway [a] and Egypt [b]

Molecular species	Phosphatidylethanolamine (% of total)		Phosphatidylcholine (% of total)	
	Norway	Egypt	Norway	Egypt
22:6/22:6	8.6	6.4	1.6	0.8
20:5/22:6	0.5	1.4	0.8	3.3
20:4/22:6	0.6	2.2	1.2	9.3
20:4/20:5	1.7	3.7	0.2	0.9
20:4/20:4	1.3	1.4	1.9	2.6
18:1/22:5	15.1	4.8	10.3	8.9
18:1/22:6 [c]	23.2	7.6	20.5	5.3
16:0/22:6	19.5	13.3	11.2	11.3
18:1/20:4	2.0	2.7	1.7	2.1
16:0/20:4	1.8	3.1	1.3	2.0
18:0/20:5	5.9	3.3	2.5	0.9
18:0/22:6	1.8	19.6	4.0	7.7
18:0/20:4	1.1	3.1	3.4	13.1
18:1/18:1	1.8	1.0	1.6	1.1
16:0/18:1	4.3	10.6	3.8	7.2
18:0/18:1	0.3	0.5	20.7	1.4
rest	10.5	15.3	13.3	22.1

[a] *Pandulus borealis* (2°C).
[b] *Parapandulus sp.* (25°C).
[c] +16:0/20:5.

Table 5
Molecular species composition of phosphatidylcholine and phosphatidylethanolamine of fish from cold-adapted (*Ophioidon elongatus*) marine and warm-adapted (*Pomadysys hasta*) marine water

Molecular species	Phosphatidylcholine (% of total)		Phosphatidylethanolamine (% of total)	
	cold-adapted [a]	warm-adapted [b]	cold-adapted [a]	warm-adapted [b,c]
22:6/22:6	2.51	0.8	2.62	1.8
20:4/20:4	2.85	2.1	1.75	0.7
18:1/20:5	2.47	1.8	11.24	0.5
18:1/22:6 [d]	22.84	9.7	25.48	6.5
16:0/22:6	21.64	32.4	8.68	23.3
18:1/20:4	2.30	2.7	3.28	0.5
16:0/20:4	2.79	10.9	5.82	1.0
18:0/20:5	8.32	5.1	20.59	5.8
18:0/22:6	5.06	8.2	8.14	7.6
18:0/20:4	0.47	1.8	1.64	26.6
18:1/18:1	1.44	0.8	0.47	2.6
16:0/18:1	10.87	5.5	0.30	7.8
18:0/18:1	0.42	1.1	0.39	0.6

[a] *Ophioidon elongatus*.
[b] *Pomadysys hasta*.
[c] Ref. [20].
[d] +16:0/20:5.

Table 6
Molecular species composition of rat liver phosphatidylcholines and phosphatidylethanolamines in relation to diet

Molecular species	Phosphatidylcholines		Phosphatidylethanolamines	
	sunflower oil	fish oil	sunflower oil	fish oil
22:6/22:6	0.17	0.36	0.68	0.27
20:4/20:4	0.84	0.33	0.44	0.60
16:1/22:6	trace	0.30	0.10	0.55
16:1/20:4	1.30	0.24	1.16	0.24
18:1/20:5	0.10	1.10	trace	0.55
18:1/22:6 [a]	0.53	6.41	0.89	7.91
16:0/22:6	7.70	19.67	15.64	11.19
18:1/22:5	trace	0.47	trace	0.22
18:1/20:4	4.28	4.72	6.00	2.80
16:0/20:4	17.40	4.28	12.76	8.35
18:0/20:5	trace	7.68	tarce	5.03
18:0/22:6	5.34	14.89	8.02	7.51
18:0/20:4	15.23	7.00	8.87	16.51
18:1/18:1	0.56	2.33	1.11	0.98
16:0/18:1	25.07	14.08	32.60	12.10
18:1/20:1	0.62	0.60	0.53	0.74
18:0/18:1	16.25	10.70	8.62	19.29
16:0/18:0	1.35	0.14	0.44	0.96
18:0/20:1	0.22	0.19	0.19	0.38
18:0/18:0	0.94	0.89	0.52	0.57

[a] +16:0/20:5.

bly this increase has been detected by 2-AS. Thus, temperature affects the molecular structure modulation profoundly at the upper half of the lipid membrane bilayer. However, we do not know why the deeper regions of the membranes remained relatively unaltered. One of the possible explanation might be that the segments below the 12th carbonic atom are fluid enough to enable the species to survive under the new thermal conditions. It is also possible that the loosely packed lipid segments will help the proteins to maintain their optimal conditions for conformation and physiological activities. In addition they can also host some new proteins involved in controlling membrane structure and function during thermal stress. This latter assumption is supposed by comparison of the so-called "homeoviscous adaptation" efficacy of an intact membrane with their extracted phospholipids molecules, these phospholipids molecules provide 40–42% adaptation, whereas the intact membrane shows a 100% homeoviscous efficacy.

From all the above observation it can be concluded that saturated-to-unsaturated fatty acid ratio plays almost no role in membrane adaptation. Reshuffling and restructuring in molecular species, mainly in PE might be an important factor in lipid membrane adaptation to temperature. Moreover, changes in the lipid molecules cannot be extrapolated to explain the thermal adaptation in an intact membrane.

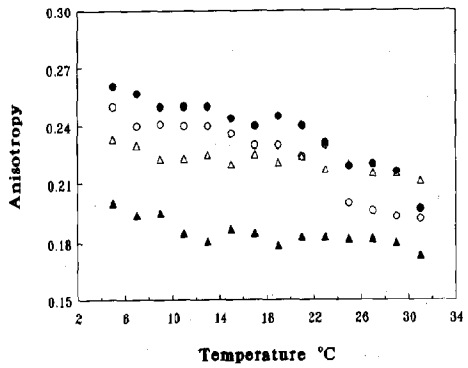

Fig. 4. Fluorescence anisotropy of 2-(9-anthroyloxy) stearic acid in rat phosphatidylcholine (PC), cold-adapted fish PC, rat PC with cold-adapted fish phosphatidylethanolamine (PE) and cold-adapted fish PC with rat PE. (●) rat PC only; (○) cold-adapted fish PC only; (▲) rat PC with cold-adapted fish PE (75% PC+25%PE) and (△) cold-adapted fish PC with rat PE (75% PC+25% PE).

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