Composition and Physical State of Phospholipids in Calanoid Copepods from India and Norway

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The fatty acid composition and physical state of isolated phospholipids obtained from marine copepods collected on the Southwest coast of India *(Calanus ssp.)* and the West coast of Norway *(Calanus finmarchicus)* were investigated to compare the adaptation of membrane lipids with seawater temperatures. Phospholipid vesicles obtained from the tropic copepods proved more rigid than those from *C. finmarchicus,* as assessed by diphenylhexatriene fluorescence polarization techniques. In each case, there were two breaks present on the fluorescence polarization vs 1/T plots, suggesting that the onset and completion of phase separation to occurred above 0 C. For the tropic copepods, the onset of phase separation roughly corresponded to the ambient water temperature, while for *C. finmarchicus* some discrepancies were observed, depending on the time of the year. Phospholipids in copepods from both habitats contained more than 50% unsaturated fatty acids, the animals from Norway containing slightly higher amounts. The data indicate an adaptation of membranes to temperature.
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Most of our knowledge concerning adaptation of the composition and physical state of membranes to temperature in higher eukariotic systems is derived from observations made on freshwater organisms (1,2). The majority of these investigations demonstrate an inverse relationship between the unsaturation of the constituent phospholipids and temperature (1,3–6), and also a varying degree of homeoviscosus response of membrane physical state to the temperature (7,8). Fatty acids are regarded as the most important factors controlling the physical state of these structures. A major difference between freshwater and marine species is the high level of long-chain polyunsaturated fatty acids in the latter. This should render their membranes more fluid. Based on the data available (2,9,10), the question remains whether they are able to control fatty acid composition according to temperature, by homeoviscosus adaptation. The oil sardine, *Sardinella longiceps,* responded by an increase in the level of docosahexaenoic acid and a decrease of saturated fatty acids when the temperature decreased from 30–31 C to 25–26 C from summer to winter (11). Absence of seasonal variation of phospholipid fatty acids was noted with two marine clam species (12) and with shrimp (13) as well as with *Porphyra yezoensis* exposed to cold for a prolonged time (14). However, in these investigations there was no direct determination of the effect of temperature on the fluidity of membrane phospholipids. In this study, the fatty acid composition and the physical state of phospholipids obtained from marine calanoid copepods collected, respectively, from tropic and temperate seas was investigated.

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MATERIALS AND METHODS

Animals. Calanus finmarchicus were collected at the West coast of Norway, at Austevoll (60°7′N, 5°13′E), and Stangvik Fjord (62°48′N, 8°26′E), on April 13, and November 26, 1984, respectively. The water temperature was near 10 C in both cases. Calanoid copepods *Calanus ssp.)* also were collected on the West coast of India. One sample originated from the coastal waters off Bombay, collected one km offshore of the Gateway of India (19°00′N, 72°45′E), on February 16, 1981. Another sample was collected at Dona Paula, Goa (15°N, 72°48′E), on January 20, 1986. The surface temperatures were 26 C and 25 C, respectively. No species were identified from the samples collected in India. The copepods originating from the Bombay area were large specimens resembling the size of *C. finmarchicus* Stage V, while those collected at Dona Paula were considerably smaller. The hauls of *C. finmarchicus* were almost 100% copepods, while those made in India were 90–95% copepods with some contaminating decapod larvae.

Analysis of lipids. The animals were fixed in chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) and frozen until transferred to the laboratory. The Folch procedure (15) was used to extract the total lipids. Phospholipids were separated by silicic acid column chromatography using chloroform to remove the neutral lipids and methanol to obtain the phospholipid fraction. The polar head group composition of the latter was determined according to Rouser et al. (16).

Phosphatidylcholine and phosphatidylethanolamine were separated for further analysis by preparative thin layer chromatography using chloroform/methanol/water (65:25:4, v/v/v) as solvent. Identification of the spots was by comparing the R_f values to those of known standards (Supelco, Bellefonte PA). The spots were detected by spraying the plates with 0.05% 8-anilino-1-naphtalene sulfonic acid in 50% methanol and viewed under UV light. Total or individual phospholipids were transmethylated in the presence of 5% HCl in absolute methanol at 80 C in sealed vials for 2.5 hr. A Hitachi 263-80 type gas chromatograph connected to a Hitachi M263 data processor was used to separate the fatty acid methyl esters. The polar phase was 10% Carbowax 20M on 100–120 mesh Supelcoport in 2 m long stainless steel columns (3 mm i.d.). The oven temperature was programed to rise linearly from 180 to 215 C, at 1 C/min. Each run was made in triplicate, and the error was not more than 1% in the case of the major fatty acids such as 22:6(n-3).

Fluorescence polarization. Phospholipid vesicles were prepared and labeled with 1,6-diphenyl 1,3,5-hexatriene (Sigma Chemical Co., St. Louis, MO) as described by Montaudon et al. (17). A Perkin Elmer Model 44A fluorescence spectrophotometer equipped with a polarization accessory and fitted with a temperature regulation

was monitored at 420 nm. Fluorescence polarization (P) was calculated from the equation, $P = I_{vv} - I_{vh}/I_{vv} + I_{vh} \cdot Z$, in which I_{vv} and I_{vh} are the fluorescence intensities measured with emission analyzer parallel or perpendicular, respectively, to the polarization of the detection system for vertically and horizontally polarized light.

RESULTS AND DISCUSSION

Figure 1 presents a Vant Hoff's representation of 1,6-diphenyl 1,3,5-hexatriene (DPH) fluorescence polarization, P, of vesicles of phospholipids prepared from marine calanoid copepods collected either in the North Atlantic or in tropic seas. Lower P-values represent more fluid structures. From these data, it may be inferred that the most fluid membranes were present in *C. finmarchicus* collected in the spring and the most rigid ones in the copepods inhabiting the tropic seas. *C. finmarchicus* sampled in early fall revealed values in between these two extremes. In addition, there are two distinct breaks in the curves on the P vs 1/T plots. The break at the higher temperature indicates onset, while that at the lower temperature indicates completion of phase separation of these phospholipids. The sea water temperature at the time of collecting *C. finmarchicus* was about 10 C but varied between 2 and 20 C during the year. The temperature in the tropic seas was about 25–26 C at the time of sample collecting and varied from 25 to 30–31 C during the year. The temperature at the onset of phase separation of phospholipids from each sample was close to the temperature at which the organism lived, except in the *C. finmarchicus* collected in the fall. However, it should be remembered that the above values for phospholipid vesicles might be modulated if other membrane constituents (proteins, sterols, etc.) also were present.

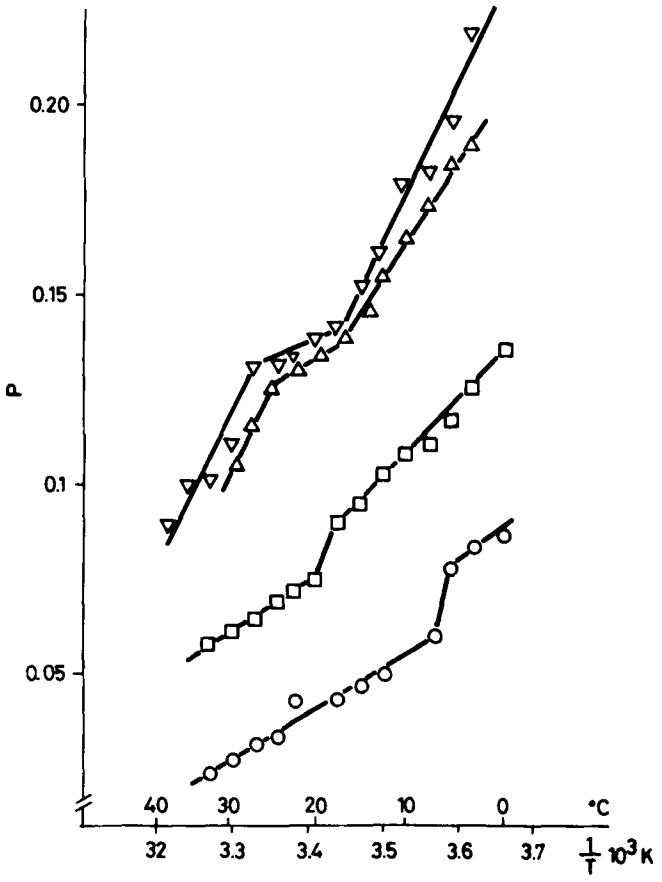

FIG. 1. Temperature dependency (1/T) of DPH fluorescence polarization (P) in phospholipids of marine calanoid copepods. *Calanus spp.* were collected at Dona Paula, Goa (—▽—) and Bombay (—△—) at the Southwest coast of India, while *C. finmarchicus* were collected

The observation that the temperature at onset of the phase separation of the membrane lipids coincides with growth temperature has been made with other poikilotherms (10,18–20), but it is not documented as a general phenomenon (7,21). Cossins and Prosser (18) reported that the onset of phase separation of phospholipids from synaptosomal membranes of arctic sculpin adapted to 0 C occurred at 5 C while that for goldfish acclimated at 5 C occurred at 10 C. It can be inferred from Figure 1 of Prosser and Cossin's paper that phospholipids of synaptosomal membranes of goldfish adapted at 25 C show phase separation around the growth temperature (18). In a current study of liver phospholipids of the carp, *Cyprinus carpio L.*, we found that the onset of phase separation of phospholipids of summer-adapted fish occurred around 25 C, while that of winter-adapted fish occurred around 6 C (unpublished observations). In earlier work on the freshwater copepod *Cyclops vicinus*, we also found that phase separation temperatures were similarly related to the actual growth temperature (10). Although the water temperature at the time of collecting *C. finmarchicus* was the same in the fall and the spring (10 C), the higher polarization value and phase separation temperature of phospholipids of the fall sample may suggest that the fall specimens retained a "summer" state in their lipids.

Whether marine species similar to *C. vicinus* can regulate the physical state of their phospholipids according to the temperature or whether they lack this property, like the freshwater crustacean *Daphnia magna*, requires further investigation. Some freshwater crustaceans and fish are exposed to fluctuation in their environmental temperature. In cases of tropic seas, this is less pronounced. Judging from the temperature range at which the phase separation occurs, it may be inferred that *C. finmarchicus* can tolerate less changes in the water temperature than the copepods in the tropic seas.

Spring-collected *C. finmarchicus* did not survive exposure to 20 C longer than two hr, and copepods collected at Dona Paula lost their swimming activity but did not die when exposed to 17 C for six hr (unpublished observations). Because the former can be regarded as a cold stenothermic and the latter as a warm stenothermic species, it is tempting to speculate that this is at least partially related to the phase behavior of their membranes.

Table 1 shows that the above differences in the phase behavior are not easily explained by the fatty acid composition of the phospholipids. An inverse relationship between environmental temperature and fatty acid unsaturation also was observed in this case. This was due

TABLE 1

Fatty Acid Composition (mol %) in Phospholipids of Calanoid Copepods

Species	*Calanus spp.*		*C. finmarchius*	
Origin	India[a]	India[b]	Norway[c]	Norway[d]
Water temperature (°C):	25	26	10	10
14:0	4.7	3.9	3.4	2.4
14:1	0.2	0.1	0.5	0.1
15:0	0.2	0.3	0.7	0.2
15:1	0.2	tr	0.1	tr
16:0	19.6	16.0	16.5	15.2
16.1	1.4	1.6	0.3	0.7
16:2	0.9	0.3	0.1	0.3
16:3	1.3	0.2	0.7	0.1
18:0	8.7	4.0	2.0	0.8
18:1	4.5	3.7	2.9	4.5
18:2	1.5	1.5	0.8	0.6
18:3	1.7	1.8	0.7	tr
18:4	0.3	2.5	1.9	0.2
20:1	tr	0.2	0.1	1.2
20:4	2.5	4.5	1.2	1.9
20:5	15.7	25.2	24.1	30.2
22:3	0.5	0.2	0.2	0.2
22:4	0.2	0.1	0.2	0.3
22:5	2.4	2.3	0.4	0.5
22:6	33.4	31.2	42.9	40.0
Sat/unsat	0.49	0.31	0.29	0.22
Total polyen(%)	55.0	66.1	71.0	73.8

[a]Bombay.
[b]Dona Paula, Goa.
[c]Spring.
[d]Fall.

TABLE 2

Composition of Phospholipids (% wt) in Calanoid Copepods

Species:	*Calanus spp.*	*Calanus finmarchius*
Origin:	India[a]	Norway[b]
Phosphatidic acid	3.6	10.1
Phosphatidylserine	3.1	8.9
Phoaphatidylinositol	3.9	6.4
Lysophosphatidyl-ethanolamine	—	5.2
Lysophosphatidyl-choline	6.8	1.1
Sphingomyelin	3.9	7.6
Phosphatidylcholine	35.6	29.3
Phosphatidyl-ethanolamine	28.9	22.3
Cardiolipin	6.8	9.8

[a]Dona Paula, Goa.
[b]Spring.

TABLE 3

Fatty Acid Composition (mol %) of Phosphatidylcholines and Phosphatidylethanolamines in Calenoid Copepods

Phospholipid	Phosphatidyl-choline		Phosphatidyl-ethanolamine	
Origin	India[a]	Norway[b]	India[a]	Norway[b]
14:0	3.6	2.5	1.0	0.5
14:1	tr	tr	tr	tr
15:0	0.4	0.1	tr	tr
16:0	20.9	10.2	20.4	24.6
16:1	1.0	1.1	0.4	0.1
18:0	2.6	0.2	12.4	2.2
18:1	6.4	2.6	2.6	4.3
18:2	1.8	0.7	1.3	1.3
18:3	2.0	0.2	0.9	0.3
18:4	2.4	1.7	0.5	1.5
20:3	tr	tr	tr	tr
20:4	4.9	5.0	6.0	3.3
20:5	21.9	42.8	14.3	9.8
22:4	0.7	tr	0.3	0.7
22:5	2.2	0.5	3.0	1.6
22:6	28.2	32.0	36.7	50.00
Sat/unsat	0.38	0.15	0.51	0.38
Total polyen(%)	62.0	82.0	61.0	67.0

[a]*Calanus ssp.*, Dona Paula, Goa.
[b]*C. finmarchicus*, spring.

the spring-collected *C. finmarchicus* and the copepods collected at Dona Paula showed similar saturated to unsaturated ratios but great differences in the phase behavior of their phospholipids. Moreover, the two samples of copepods from the tropic seas showed differences in phospholipid fatty acid compositions as well as in the saturated to unsaturated fatty acid ratios, although the P-values and the phase separation temperatures were almost identical (Fig. 1). Thus, it is highly probable that control occurs at a level beyond the overall distribution of fatty acids in phospholipids. Table 2 shows that the phospholipids of copepods from tropic seas were poorer in sphingomyelin and phosphatidic acid, and richer in phosphatidylethanolamine than those in *C. finmarchicus*. Phosphatidylcholines in spring-collected *C. finmarchicus* contained more polyunsaturated acids (82% vs 62%) and had a lower saturated-to-unsaturated fatty acid ratio (0.15 vs 0.38) than those of the tropic copepods (Table 3). Even though phospholipids were not separated according to molecular-species composition, one could expect that diunsaturated phospholipids would be present whenever the level of total unsaturated fatty acids exceeds 50 mol %. As shown in Table 3, the phosphatidylcholines and phosphatidylethanolamines were richer in diunsaturated phospholipids than were the phospholipids of copepods in the tropic seas (32% vs 12% and 17% vs 11%, respectively), of 1-palmitoyl,2-docosahexaenoyl phosphatidylcholine is about −10 C (22) and that of diunsaturated molecules is even lower, phospholipids of marine copepods should exhibit lower phase-separation temperatures than those observed. Phospholipids of these copepods behave similarly to those of the bovine retinal rod outer segment membranes. Although the latter are as rich in polyenes as the phospholipids of copepods investigated here, they too contain fair amounts of supraenes (dipolyunsaturated

It has been proposed that this results from a precise balance between disaturated and diunsaturated phospholipid molecules (25). The differences demonstrated in physical parameters of phospholipid vesicles indicate an adaptation of membrane physical states to temperatures.

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