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POSITIONAL AND SPECIES ANALYSIS OF MEMBRANE PHOSPHOLIPIDS EXTRACTED FROM GOLDFISH ADAPTED TO DIFFERENT ENVIRONMENTAL TEMPERATURES

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SUMMARY

1. The proportion of ethanolamine phosphoglycerides in microsomal fractions of goldfish intestine increases at low environmental temperatures. The fatty acyl composition also changes, the proportion of $C_{22:6}$ and $C_{20:4}$ fatty acids increasing in positions 1 and 2 and position 2 respectively. The proportion of $C_{16:0}$ and $C_{18:0}$ fatty acids falls in position 1 and there is an apparent switch of $C_{18:1}$ and $C_{20:1}$ fatty acids from position 2 to position 1.

2. The proportion of choline phosphoglycerides does not depend on the previous environmental temperature of the fish. Temperature-dependent changes in fatty acyl composition in positions 1 and 2 take place in a way similar to that described for ethanolamine phosphoglycerides, but in this case $C_{22:6}$ substitution is confined to position 2.

3. Choline phosphoglycerides have been further separated into 7 different molecular species. The amounts of species 3 to 7 increase and the amount of species 2 decreases at low adaptation temperature. These changes only account for part of the total change in fatty acyl composition. The remaining changes occur by chain substitution within species.

4. Present results show temperature adaptation to be highly complex, involving both quantitative and qualitative changes in different phospholipids. The possible physiological significance of these changes are discussed together with the effects these changes might have on cholesterol-phospholipid interactions.

INTRODUCTION

It has been shown previously that the fatty acyl composition of goldfish intestinal phospholipids can be modified by a change in environmental temperature [1, 2]. The proportion of unsaturated to saturated fatty acyl chains increases as the environmental temperature falls. The assumption is that these changes increase membrane fluidity and that this is essential to maintain normal cellular function at low environmental temperatures.

A more precise definition of membrane fluidity can be obtained both through the use of membrane probes and by NMR analysis of different codispersions of phospholipids with cholesterol. Using probes it can be shown that a flexibility gradient exists along the acyl chains, their rotational movement increasing with distance from the polar head groups [3]. This flexibility gradient, which depends mainly upon the degree of unsaturation of the individual acyl chains [4], can be partly suppressed by the presence of cholesterol. NMR analysis shows cholesterol to form complexes with dipalmitoyl phosphatidylcholine and these complexes can form larger clusters within bilayers [5]. A comprehensive review of cholesterol/phospholipid interactions has been published recently by Jain [6].

The degree of cholesterol suppression depends upon the efficiency with which this substance interacts with phosphatidylcholine. This is determined both by the shape and position of the different acyl chains [7]. A knowledge of cholesterol content and a positional analysis of these acyl chains are therefore needed before attempting to understand how these complex interactions operate during adaptation to different environmental temperatures. The present work was undertaken to provide this basic information.

MATERIALS AND METHODS

Animals

Goldfish measuring 5–7 inches, purchased from Robinsons Fisheries, South Woodford, London, were kept in aerated water at 6 or 30 °C for at least 4 weeks before use. Warm-adapted fish were fed twice daily. Cold-adapted fish, which were generally much less active, were fed on alternate days.

Preparation of membrane fractions

Goldfish were killed, their anterior intestines removed, and the separated mucosa homogenized in 10 vol. (v/wt.) of ice-cold 0.25 M sucrose containing 5 mM EDTA, 60 mM histidine and 0.1 % sodium deoxycholate, pH 7.1. The pooled homogenate was centrifuged for 15 min at $10\,000 \times g$ and the pellet discarded. The supernatant was then centrifuged at $20\,000 \times g$ for 60 min and the microsomal pellet used for lipid analysis. Previous enzyme assays on separated fractions of goldfish mucosal homogenates showed the $20\,000 \times g$ fraction to have the highest specific activity for both alkaline phosphatase and Mg^{2+}- and $(Na^+ + K^+)$-activated adenosine triphosphatase activity [8]. Alkaline phosphatase is a marker enzyme for brush borders and $(Na^+ + K^+)$-activated adenosine triphosphatase is found mainly in the baso-lateral membranes. Brush borders become disrupted in the medium used for homogenization and small fragments then centrifuge down along with baso-lateral membranes.

Analytical procedures

Microsomal pellets were divided into two, half being used for determination of protein [9] and half for determination of lipid. Total lipid, extracted with 30 vol. of chloroform/methanol (2 : 1, v/v) [10] was washed according to Folch, dried, and the total recovery determined by weight. Protein and lipid contents of membranes were then represented as mg of material recovered per g mucosal scrapings. Phospholipids

and cholesterol were separated by thin-layer chromatography, using silica gel H in chloroform/methanol/7M ammonia (46 : 18 : 3, v/v/v) according to the method of Abramson and Blecher [11]. The amount of phospholipid present was determined by phosphate analysis [12]. Cholesterol was determined colorimetrically using the method of Huang et al. [13]. The amount of neutral lipid present was determined as the difference in weight between total lipid and phospholipid plus cholesterol recovered from thin-layer chromatography.

Choline and ethanolamine phosphoglycerides, separated as described above, were used for positional analysis. Acyl chains in position 2 were removed by incubation with *Crotalus adamanteus* or *Naja naja* venom at 37 °C for a period of 2–3 h at pH 7.4 in the presence of 10 mM Ca^{2+}. Complete hydrolysis of material was verified by subsequent thin-layer chromatography on silica gel G using chloroform/methanol/water (65 : 25 : 4, v/v/v) [14]. Only two spots, identified as lysophospholipids and fatty acids, appeared on these plates. The separated lysophospholipids and fatty acids were finally transmethylated using 5 % anhydrous HCl in MeOH [15] for analysis by gas-liquid chromatography (Pye dual flame ionisation detector, model 104). These analyses were then compared with the fatty acyl composition of unhydrolysed material determined separately.

Choline phosphoglycerides were fractionated according to the degree of unsaturation using silica gel H impregnated with $AgNO_3$ [14]. The absolute amounts of each species was then determined by phosphate analysis and the fatty acyl composition measured as described above.

Material kept prior to analysis was stored in chloroform at −20 °C under N_2. All lipids were identified against known standards.

Materials

Lipid standards were obtained from the Sigma Chemical Co., London, and Supelco Inc., Bellefonte, Pa. 16823. Materials for thin-layer chromatography came from Merck, Darmstadt, F.D.R. All other reagents were of AR grade.

RESULTS

Preparations used in this work consist of ruptured brush border membranes mixed with baso-lateral membranes from goldfish intestinal mucosa. Preliminary experiments were carried out to see whether adaptation of goldfish to two widely different

TABLE I

PROTEIN AND LIPID ANALYSES OF MICROSOMAL FRACTIONS PREPARED FROM POOLED MUCOSAL SCRAPINGS TAKEN FROM THE INTESTINES OF 5–10 GOLDFISH ADAPTED TO 6 OR 30 °C.

Values give means ± S.E. (no. of determinations given in parentheses).

	Protein (mg/g mucosa)	Lipid (mg/g mucosa)
30 °C-adapted	5.91 ± 0.83 (5)	3.63 ± 0.39 (4)
6 °C-adapted	5.13 ± 0.42 (5)	4.26 ± 0.58 (4)

environmental temperatures would have any effect on the gross yield or composition of these membranes. Table I shows the amounts of protein and lipid recovered from 1 g of starting material. The yield of protein and lipid remained independent of the previous adaptation temperature of the fish. The protein to lipid ratio was 1.6 for the 30 °C-adapted and 1.2 for the 6 °C-adapted membranes, but this difference was not found to be statistically significant.

Further fractionation of the total lipid extract into phospholipid, neutral lipid and cholesterol fractions produced the results shown in Table II. The amount of neutral lipid present in the original membranes was obtained by adding the amount of

TABLE II

ANALYSIS OF GOLDFISH INTESTINAL MICROSOMAL LIPID

Chloroform-methanol extracts were separated into phospholipids and cholesterol using thin-layer chromatography as described in the text. The amount of neutral lipid present was determined by weight difference. Values are given per 100 mg total lipid. Each experiment was carried out on lipid obtained from microsomes prepared from the pooled mucosal scrapings of from 5 to 10 fish.

Experiment	Phospholipid (mg)	Neutral lipid (mg)	Cholesterol (mg)
30 °C-adapted			
1	64.7	20.6	14.7
2	68.8	14.9	16.3
3	59.5	22.6	17.9
4	40.5	46.1	13.3
Mean	58.4±6.3	26.1±6.9	15.6±1.0
6 °C-adapted			
1	53.7	29.6	16.8
2	63.2	20.3	16.8
Mean	58.5	25.0	16.8

TABLE III

ANALYSIS OF DIFFERENT PHOSPHOLIPIDS EXTRACTED FROM MICROSOMAL FRACTIONS PREPARED FROM THE INTESTINES OF GOLDFISH ADAPTED TO 6 OR 30 °C

U_1 and U_2, unidentified phospholipids, possibly cardiolipin and lysolecithin respectively; PE, PC, PS and PI, phosphoglycerides of ethanolamine, choline, serine and inosine respectively, SM, sphingomyelin. Each value gives the mean±S.E. of 4 determinations. Each determination on 30°C-adapted membranes was made on material pooled from 5 fish; that carried out on 6 °C-adapted membranes used material pooled from 8 to 10 fish

Phospholipid	30 °C-adapted	6 °C-adapted
U_1	7.2±0.7	4.0±0.2
PE	18.2±2.3	26.0±2.4
PC	48.2±0.9	45.4±2.2
PS+PI+SM	23.1±0.9	21.5±0.9
U_2	3.2±0.5	3.0±0.4

phospholipid recovered to that of cholesterol, then subtracting this value from the weight of starting material. This assumes 100 % recovery of phospholipids and cholesterol from thin-layer chromatography. Just over half of the total lipid consisted of phospholipids, the rest being divided between neutral lipid and cholesterol in a ratio of about 2 : 1. These proportions were independent of the previous environmental temperature of the fish. Further analysis of the fractionated phospholipids produced the results summarized in Table III. About half the phospholipids present consisted of choline phosphoglycerides. This result, which applied to membranes prepared from both 6 and 30 °C-adapted fish, confirmed that found previously [1]. The proportion of ethanolamine phosphoglycerides fell from 26 to 18 % of the total phospholipid present, on changing the environmental temperature from 6 to 30 °C. This decrease is compensated mainly by an increase in U_1 and phosphatidylcholine fractions. This is contrary to previous work, where the proportion of ethanolamine phosphoglycerides was found to remain independent of the adaptation temperature [1].

The fatty acyl composition of goldfish intestine ethanolamine phosphoglycerides has been shown to depend on the previous adaptational temperature of the fish [1]. Present experiments show how these temperature-dependent changes distribute between the 1- and 2-position on the phospholipid molecules. These results are summarized in Table IV. The fatty acyl composition of ethanolamine phosphoglycerides, determined before hydrolysis, shows increases in the amounts of long chain, unsaturated, fatty acyl groups on changing the environmental temperature from 30 to

TABLE IV

FATTY ACYL CHAIN COMPOSITION OF ETHANOLAMINE PHOSPHOGLYCERIDES EXTRACTED FROM MICROSOMAL FRACTIONS PREPARED FROM INTESTINES OF GOLDFISH FULLY ADAPTED TO 6 OR 30 °C

Conditions for carrying out positional analysis are as described in the text. Values represent means obtained from material prepared from 5+5 fish (30 °C-adapted) and 8+10 fish (6 °C-adapted).

Fatty acyl group (% total)	Ethanolamine phosphoglycerides					
	30 °C-adapted			6 °C-adapted		
	Total	Chain 1	Chain 2	Total	Chain 1	Chain 2
$C_{16:0}$	17.5	21.5	13.4	12.2	17.3	7.1
$C_{16:1}$	0.6		1.2	1.2	2.1	0.3
$C_{18:0}$	39.7	74.9	4.4	21.9	37.4	6.4
$C_{18:1}$	8.9		17.8	7.6	7.6	7.6
$C_{18:2}$	9.5	3.5	15.4	6.7	3.1	10.2
$C_{18:3}$	0.2		0.4	3.2	4.9	1.5
$C_{20:1}$	0.7		1.4	8.2	13.9	2.4
$C_{20:2}$	2.2		4.4	2.2	3.3	1.0
$C_{20:3}$	1.4		2.8	0.3		0.6
$C_{20:4}$	4.1		8.2	6.9		13.7
$C_{20:5}$	0.2		0.4	1.7		3.4
$C_{22:3}$	0 6		1.2	1.2		2.3
$C_{22:4}$	1.9		3.8	2.7		5.4
$C_{22:5}$	0.4		0.8	1.3		2.6
$C_{22:6}$	12.1		24.2	22.4	10.2	34.5

6 °C. This applies to $C_{20:4}$, $C_{20:5}$, $C_{22:3}$, $C_{22:4}$, $C_{22:5}$ and $C_{22:6}$ acyl groups. These changes are compensated for mainly by a fall in the amount of $C_{16:0}$ and $C_{18:0}$ acyl groups present. It was shown in Table III that ethanolamine phosphoglyceride, a phospholipid of relatively high transition temperature, increases on adaptation to a cold environment. Compositional changes within the different species of ethanolamine phosphoglycerides will, however, tend to lower the transition temperature of this phospholipid at low adaptational temperatures. In this respect the contribution of the head group and chain length to the fluidity of the membrane is much less than that of double bonds [16].

Positional analysis of the warm-adapted ethanolamine phosphoglycerides shows the 1-position to be relatively homogeneous ($C_{16:0}$, $C_{18:0}$ and $C_{18:2}$). This becomes more complex on adaptation to a low environmental temperature. The proportion of $C_{16:0}$, $C_{18:0}$ and $C_{18:2}$ fatty acyl groups falls, to be replaced mainly by $C_{18:1}$, $C_{20:1}$ and $C_{22:6}$ acyl groups. The major importance of these three fatty acyl groups for position-1 substitution is even more noticeable for choline phosphoglycerides (see below). The 2-position of the ethanolamine phosphoglycerides of 30 °C-adapted membranes also changes on adaptation to 6 °C, fatty acyl groups $C_{16:0}$, $C_{18:1}$ and $C_{18:2}$ being supplemented by the longer chain, polyunsaturated fatty acids.

These experiments show, firstly, an ability of the 1-position as well as the 2-position to accept long chain highly unsaturated fatty acyl groups on adaptation

TABLE V

FATTY ACYL CHAIN COMPOSITION OF CHOLINE PHOSPHOGLYCERIDES EXTRACTED FROM MICROSOMAL FRACTIONS PREPARED FROM INTESTINES TAKEN FROM GOLDFISH FULLY ADAPTED TO 6 OR 30 °C

Conditions for carrying out positional analysis are as described in the text. Values represent mean obtained from material prepared from 5+5 fish (30 °C-adapted) and 8+10 fish (6 °C-adapted)

Fatty acyl group (% total)	Choline phosphoglycerides					
	30 °C-adapted			6 °C-adapted		
	Total	Chain 1	Chain 2	Total	Chain 1	Chain 2
$C_{16:0}$	33.1	50.9	15.3	23.9	36.3	11.6
$C_{16:1}$				1.9		3.9
$C_{18:0}$	22.7	43.9	1.4	10.5	19.9	2.0
$C_{18:1}$	8.5		16.9	16.5	22.4	10.6
$C_{18:2}$	15.9	5.1	26.7	13.6	2.8	24.4
$C_{18:3}$	0.2		0.3	0.7		1.3
$C_{18:4}$	trace		trace	trace		trace
$C_{20:1}$	0.6		1.1	4.2	6.7	1.7
$C_{20:2}$	3.0		6.0	1.0		1.9
$C_{20:3}$	3.2		6.3	1.6		3.2
$C_{20:4}$	4.4		8.7	7.6		15.2
$C_{20:5}$	0.3		0.5	1.5		3.0
$C_{22:3}$	0.3		0.5	0.6		1.1
$C_{22:4}$	0.8		1.6	1.2		2.3
$C_{22:5}$	0.6		1.1	1.2		2.3
$C_{22:6}$	6.4		12.8	12.9	11.8	13.9

to a cold environment. They also demonstrate that acyl groups such as $C_{18:1}$, confined to the 2-position in warm-adapted membranes, can appear in the 1-position on adaptation, without changing its overall proportion in the unhydrolysed phospholipid (7.6 and 8.9 % of the total for 6 and 30 °C-adapted membranes respectively). The position of the fatty acyl group on the phospholipid molecule, as well as its length and degree of unsaturation, could be important in determining the physiological properties of membranes at different environmental temperatures.

The fatty acyl composition of choline phosphoglycerides, prepared from fish previously adapted to 6 or 30 °C, changes in a similar way to ethanolamine phosphoglycerides. These results are shown in Table V. Chains longer and/or more unsaturated than $C_{20:4}$ increase their relative proportion, the difference being accounted for mainly by a decrease in the amounts of $C_{16:0}$ and $C_{18:0}$ acyl groups present. Positional analysis of 30 °C-adapted choline phosphoglycerides shows the 1-position to consist of $C_{16:0}$, $C_{18:0}$ and $C_{18:2}$ acyl groups, a situation similar to that found for ethanolamine phosphoglycerides. Adaptation to 6 °C reduces the proportion of these acyl groups in favour of $C_{18:1}$, $C_{20:1}$ and $C_{22:6}$. This again corresponds to results found for ethanolamine phosphoglycerides, the only difference being that small amounts of other acyl groups, present in the 1-position of 6 °C-adapted ethanolamine phosphoglycerides, are absent from the choline phosphoglycerides. Alterations in position-2 also take place on moving from a warm to a cold environment. These consist mainly in an increase in the number of longer chain acyl groups ($C_{20:4}$ and above) with a corresponding decrease in the amount of $C_{16:0}$ and $C_{18:0}$ present.

It is possible to separate different molecular species of choline phosphoglycerides on $AgNO_3$ impregnated silica gel to see whether changes in fatty acyl composition correspond to a change in the amount of a single phospholipid species or whether they represent changes taking place within individual species of choline phosphoglycerides. The amounts of seven different species separated in this way, are compared for two different adaptation temperatures in Table VI. Changing from a warm to a cold environment increases the amount of species 3, 4, 5, 6 and 7 to the

TABLE VI

PHOSPHATE ANALYSIS OF CHOLINE PHOSPHOGLYCERIDES SEPARATED INTO DIFFERENT SPECIES BY CHROMATOGRAPHY ON $AgNO_3$ IMPREGNATED SILICA GEL

The experimental conditions for separation and subsequent phosphate analysis of different choline phosphoglycerides are as described in the text. The processed material derived, in each case, from the pooled mucosal scrapings of 10 fish. Species 1 represents the most and species 7 the least saturated species of choline phosphoglyceride present.

Species	Choline phosphoglycerides (% total)	
	30 °C-adapted	6 °C-adapted
1	5.9	7.9
2	46.6	8.8
3	11.1	21.5
4	8.9	12.4
5	7.5	12.2
6	13.1	27.3
7	6.7	9.9

TABLE VII

FATTY ACYL COMPOSITION OF CHOLINE PHOSPHOGLYCERIDES SEPARATED INTO DIFFERENT SPECIES BY CHROMATOGRAPHY ON $AgNO_3$ IMPREGNATED SILICA GEL.

The material used for these analyses was that referred to in Table VI. Trace amounts of material present (< 2 % of the total) have not been tabulated unless the percentage of that particular fatty acyl group exceeded 2 % in one of the other isolated species. Material from fish fully adapted to 30 and 6 °C are indicated.

Fatty acyl group	Temperature (°C)	Fatty acyl composition of separated choline phosphoglycerides (% total in each species)						
		1	2	3	4	5	6	7
$C_{16:0}$	30	32.1	36.7	24.3	26.2	34.5	35.8	20.9
	6	26.4	31.7	25.6	19.9	26.6	28.2	12.6
$C_{16:1}$	30	3.5	1.8	2.2	2.6	2.8	1.3	4.6
	6	8.3	5.1	2.0	3.2	2.4	1.9	6.3
$C_{18:0}$	30	21.4	10.2	11.4	10.0	14.0	12.8	8.9
	6	16.2	6.1	8.3	5.6	8.2	5.9	4.2
$C_{18:1}$	30	17.8	17.9	5.6	13.5	12.9	6.7	18.4
	6	25.0	17.0	5.8	17.3	4.9	6.7	18.2
$C_{18:2}$	30	14.2	27.3	15.7	6.8	13.6	3.3	11.6
	6	4.2	30.7	14.6	4.3	5.4	1.9	11.9
$C_{18:3}$	30	3.5		0.9	1.3	1.4		2.1
	6	1.4		0.6	0.3	0.1	0.1	0.7
$C_{18:4}$	30			2.9	3.2	2.8		
	6	9.3	0.1	7.6		1.4	0.5	2.0
$C_{20:1}$	30	3.6	1.5	0.4	0.3		0.8	2.4
	6		2.5	1.2	2.3	0.9		2.0
$C_{20:2}$	30		1.3	3.3	0.6		0.4	
	6		1.2	0.3	0.9	0.5	0.3	1.4
$C_{20:3}$	30		1.7	7.1	1.6		0.8	4.0
	6		1.8	5.2	0.7	0.6	0.3	2.0
$C_{20:4}$	30			20.6	18.4	6.5	1.0	
	6			23.6	23.6	5.5	0.8	
$C_{20:5}$	30				2.2	0.7	0.3	
	6					17.8	5.2	18.9
$C_{22:4}$	30			3.5	1.6	1.4	0.4	23.9
	6			3.3	2.5			1.4
$C_{22:5}$	30				8.1	2.8	2.0	
	6				9.7	21.7	2.7	4.8
$C_{22:6}$	30						31.6	
	6						40.7	12.6

general detriment of species 2. These results should be considered alongside analyses of fatty acyl chain composition, carried out on the same seven species of choline phosphoglycerides. These results are shown in Table VII. Considering, first, only the analyses of choline phosphoglycerides from 30 °C-adapted fish, it can be seen that most of the $C_{20:4}$ fatty acyl groups reside in species 3 and 4 and that species 6 is the only choline phosphoglyceride containing $C_{22:6}$ chains. Increases in the amounts of these species will increase the average chain length and degree of unsaturation of constituent fatty acyl groups. The same applies to species 5 and 7, which contain longer chain, more unsaturated, fatty acyl groups than species 2, the percentage com-

position of which falls from 46.6 to 8.8 % of the total choline phosphoglycerides on adaptation from 30 to 6 °C. These results show that species substitution can account for at least part of the total change in fatty acyl group composition, seen to take place on adaptation to different environmental temperatures. Species 1 has an unusual acyl composition for its position on thin-layer chromatography, containing a larger proportion of unsaturated acyl groups than expected. This could be explained by the presence of a plasmalogen form of phosphatidylcholine in this fraction. There was not enough material to test this point further.

Additional changes take place within species as the fish adapt from 30 to 6 °C. Species 5, which is not particularly rich in acyl group $C_{22:5}$ at an adaptation temperature of 30 °C, contains 21.7 % of this fatty acid at 6 °C (Table VII). Species 7, which contains no $C_{22:6}$ fatty acyl groups at an adaptation temperature of 30 °C, contains 12.6 % of these groups at 6 °C. These intraspecies changes in fatty acyl group composition have an additional effect in increasing the average chain length and degree of unsaturation of mixed choline phosphoglycerides.

It is possible to see more clearly how these changes in fatty acyl composition come about by considering the significant, i.e. > 3 %, temperature-dependent changes in fatty acyl composition in individual species alongside results of positional analysis carried out on mixed choline phosphoglycerides. Temperature-dependent changes within species are shown in Table VIII. All species show falls of similar magnitude in the proportion of $C_{16:0}$ and $C_{18:0}$ fatty acyl groups present. Positional analysis has shown these changes to be virtually confined to the 1-position (Table V). The substituent fatty acyl groups can vary from $C_{16:1}$ to $C_{22:6}$ (Table VIII). Taking the longer chain fatty acyl groups first ($C_{20:4}$ and $C_{20:5}$), it can be seen from positional analysis that these groups exist only in position-2 of 30 °C-adapted fish. Adaptation to the cold leads to an increase of $C_{20:4}$ and $C_{20:5}$ in the 2-position. This increase in

TABLE VIII

SUMMARY OF COMPOSITIONAL CHANGES TAKING PLACE IN DIFFERENT SPECIES OF CHOLINE PHOSPHOGLYCERIDES FOLLOWING A CHANGE IN ENVIRONMENTAL TEMPERATURE OF GOLDFISH FROM 30 TO 6 °C

Small differences in composition (< 3 % of the total) have not been tabulated.

Fatty acyl group	Percentage change in species						
	1	2	3	4	5	6	7
Always negative							
$C_{16:0}$	− 5.7	− 5.0		−6.3	− 7.9	− 7.6	− 8.3
$C_{18:0}$	− 5.2	−4.1	−3.1	−4.4	− 5.8	−6.9	− 4.7
Usually positive							
$C_{16:1}$	+ 4.8	+ 3.3					
$C_{18:1}$	+ 7.2			+ 3.8			
$C_{18:2}$	−10.0	+ 3.4			− 8.2		
$C_{18:4}$	+ 9.3		+ 4.7	−3.2			
$C_{20:4}$			+ 3.0	+ 5.2			
$C_{20:5}$					+ 17.1	+ 4.9	+ 18.9
$C_{22:4}$							+ 22.5
$C_{22:6}$						+ 9.1	+ 12.6

position-2 unsaturation cannot be balanced directly by a fall in the amounts of $C_{16:0}$ and $C_{18:0}$ present in the 1-position. Fatty acyl groups $C_{18:1}$ and $C_{20:1}$, confined entirely to position-2 in warm-adapted choline phosphoglycerides, appear in the 1-position on adaptation to the cold and the proportion of $C_{18:1}$ falls in position-2. This can be seen, intuitively, as a way of making room for the more unsaturated fatty acyl groups, but the biochemical pathways responsible for these changes are entirely unknown.

The fatty acyl group $C_{22:6}$ does not increase its proportion in position-2 on adaptation to 6 °C (Table V), although species 6 and 7 show a large increase in the amount of this fatty acid present (plus 9.1 and 12.6 % respectively, Table VIII). Substitution here appears to be directly into position-1 in exchange for $C_{16:0}$ and $C_{18:0}$ fatty acyl groups.

The proportions of $C_{16:1}$, $C_{18:1}$, $C_{18:2}$ and $C_{18:4}$ all increase in the more saturated species of choline phosphoglycerides on adaptation to 6 °C and there are one or two, apparently anomalous, decreases in the amounts present. It is difficult to make any definite conclusion concerning substitution of these shorter fatty acyl chains with the exception of $C_{16:1}$ which appears to substitute in position-2 in a way similar to that seen for $C_{20:4}$ and $C_{20:5}$.

DISCUSSION

The primary function of a cell membrane is to separate two different environments, contact between the two being maintained through the presence of specific carrier-mediated pathways. Yet all membranes allow a certain amount of passive diffusion to take place. Changing the environmental temperature can be expected to produce immediate effects on passive as well as active transport of materials across membranes. Subsequent adaptation might be expected to further modify both types of process to produce a new situation, optimal for cell function at the new temperature. Part of this adaptation consists of modifying the fatty acyl composition of membrane phospholipids. Present results show this change to be unusually complex, expected increases in long chain unsaturated fatty acyl groups in position 2 being accompanied by unexpected changes in position 1 and by an apparent change in the relative preference of esterifying enzymes for different acyl groups.

The overall increase in degree of unsaturation will, by itself, increase membrane fluidity. The selective positioning of certain fatty acyl groups in phospholipids probably results in a further disorganization of membrane structure at low adaptation temperatures. It has been shown that cholesterol condenses expanded films of phosphatidylcholines, maximal effects being produced with 1-saturated, 2-unsaturated molecules [17]. Condensation of $C_{18:0}$ $C_{18:2}$ is much greater than that found with $C_{18:2}$ $C_{18:2}$ or $C_{18:2}$ $C_{18:0}$. If this finding is applicable to other fatty acyl groups, one would predict that the presence of $C_{18:1}$, $C_{20:1}$ and $C_{22:6}$ acyl groups in the 1-position of phospholipids extracted from cold-adapted membranes would further increase membrane fluidity by limiting condensation with cholesterol.

The implied suggestion so far has been that these changes are organized by the fish for its own benefit, but there are other ways in which these changes can be brought about. Reducing the environmental temperature both increases the oxygen tension and decreases the food intake of goldfish. Either of these variables could be

responsible for an overall change in fatty acyl composition. Short-term food deprivation can inhibit the amount of saturated fatty acid synthetase in rat liver and hence reduce the availability of these fatty acids for phospholipid synthesis [18]. The situation is complicated here since starvation also inhibits microsomal desaturating enzyme in the same tissue [19]. However, the desaturase enzymes are normally limited by the supply of oxygen. They would be expected to operate more efficiently at low environmental temperatures where the oxygen tension is greater [20]. Although this might have some influence on the overall composition of fatty acyl groups within different phospholipids, it hardly provides a complete explanation for the selective substitutions seen to take place both within and between different species of phosphatidylcholines. The intestinal mucosa has to provide nutrients for the rest of the organism as well as maintain its own integrity at different environmental temperatures. One would predict that such as important function would be at least partly under the control of the organism itself.

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