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DIURNAL VARIATION IN MEMBRANE LIPID COMPOSITION OF SONORAN DESERT TELEOSTS

BY CYNTHIA CAREY AND JEFFREY R. HAZEL

Department of Evolutionary, Population and Organismic Biology, University of Colorado, Boulder, CO 80309 and *Department of Zoology, Arizona State University, Tempe, AZ 85287, USA*

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Summary

Phospholipid molecular species and headgroup compositions were determined for sarcoplasmic reticular and microsomal membranes in two species of Sonoran desert cyprinid fish (*Agosia chrysogaster* Girard and *Notropis lutrensis* Girard) trapped in an isolated pool of a desert stream. Fish populations were sampled in the cool of the morning (water temperature 21–21·9°C for *Agosia* and 22–26°C for *Notropis*) and 7–11 h later in the heat of the afternoon (water temperature 34·2°C) to determine whether membrane lipid composition varied with sampling time and thermal exposure. The composition of sarcoplasmic reticular membranes did not vary significantly through the day. In contrast, the molecular species composition of muscle microsomes (a mixture of endoplasmic reticulum and plasma membranes) changed markedly from the cool of the morning to the heat of the afternoon in both species. Adjustments were particularly striking in phosphatidylcholine (PC). For example, in *Agosia*, the ratio of saturated-to-unsaturated species of PC, the unsaturation index (the average number of double bonds per molecular species) and the proportion of diunsaturated molecular species decreased from 7·48 to 0·77, from 1·36 to 0·35 and from 7·23% to 2·46%, respectively, between the morning and afternoon samples. Similar, but less dramatic, changes were noted for phosphatidylethanolamine (PE) in both species, and for phosphatidylinositol (PI) in *Notropis*. In addition, microsomal membranes of *Agosia* exhibited a significant reduction in the proportion of PE (from 12·1 to 2·7%) and a corresponding increase in the proportion of PC between the morning and afternoon samples. These patterns of change in membrane lipid composition are in a direction consistent with thermal compensation of membrane function and suggest that rapid adjustments in the lipid composition of biological membranes may stabilize membrane structure against substantial diurnal fluctuations in temperature.

Key words: temperature, sarcoplasmic reticulum, microsomal membranes, phospholipid composition, phospholipid molecular species, Sonoran desert, ectotherms, homeoviscous adaptation.

Introduction

The remodelling of biological membrane lipid composition in response to changes in temperature is perhaps the most recurrent cellular adaptation of poikilotherms to alterations in growth temperature and is believed to be responsible for thermal compensation of membrane function (Hazel, 1988). Rates of change in the fatty acid composition of membrane lipids suggest that a period of acclimation, varying in duration from one (warm acclimation) to several weeks (cold acclimation), is required for such homeoviscous adaptation (Sellner & Hazel, 1982; Cossins *et al.* 1977; Hagar & Hazel, 1985). For this reason, the restructuring of membrane lipid composition has been regarded, primarily, as an adaptation to cope with seasonal fluctuations in temperature.

Recent studies with rainbow trout indicate that some aspects of membrane structure change within hours of a variation in water temperature (Hazel & Landrey, 1988*a*,*b*). Specifically, the ratio of phosphatidylcholine (PC) to phosphatidylethanolamine (PE) in both gill (Hazel & Carpenter, 1985) and renal plasma membranes (Hazel & Landrey, 1988*a*) varies significantly in response to a temperature change of only a few degrees centigrade. Certain aspects of the composition of phospholipid molecular species also respond rapidly to changes in water temperature (Hazel & Landrey, 1988*b*). In fact, adjustments in both headgroup and molecular species composition precede alterations in acyl chain composition (unsaturation) during thermal acclimation (Hazel & Landrey, 1988*b*). These results suggest that unique adjustments to the lipid composition of biological membranes may permit some degree of compensation for the rapid changes in temperature experienced diurnally by many poikilotherms.

If mechanisms do exist for the adaptation of membrane function to rapid changes in temperature, such mechanisms should be evident in poikilotherms inhabiting environments characterized by large and rapid thermal fluctuations. Sonoran desert streams and the ephemeral, isolated pools they give rise to are examples of such environments. In Arizona, at elevations below 1000 m, air temperatures may vary diurnally by as much as 30 °C in spring and autumn, and water temperatures may vary by as much as 20 °C in shallow streams (Deacon & Minckley, 1974).

The present study was undertaken to determine whether fish species inhabiting shallow streams of the lower Sonoran desert possess the capacity to modify the lipid composition of their cellular membranes in response to diurnal variations in water temperature. Two species were selected for study. The longfin dace, *Agosia chrysogaster*, is a cyprinid fish native to the deserts of the American southwest. As streams desiccate, these fish may become trapped in tiny, shallow pools and, in the terminal stages of watercourse drying, persist alive beneath saturated mats of algae and debris; fish emerge at night, when water flow is resumed, to swim and feed in a few millimetres of water (Deacon & Minckley, 1974). The red shiner, *Notropis lutrensis*, in contrast, is an introduced cyprinid species which is highly resistant to environmental extremes within its native range (Cross, 1967).

Materials and methods

Animals and experimental design

Agosia chrysogaster and *Notropis lutrensis* (approximately 80 individuals of each species) were captured by seine from Sycamore Creek (near Sunflower, AZ) in the Tonto National Forest, Maricopa County, Arizona, and stocked into a small (5 m × 20 m × 10 cm deep), isolated pool adjacent to the creek at 718 m elevation. The stocking supplemented existing fish in the pool and was done to facilitate rapid capture for sampling and the accurate estimation of body temperature (temperatures in the pool were more homogeneous than those in the stream). Fish remained in the pool for 2 days prior to sampling. On 14 May 1984 samples were collected at two times: approximately half the *Agosia* were captured between 05.40 and 07.30 h (at a pond temperature of 21–21·9 °C and an air temperature of 20 °C) and the *Notropis*, subsequently, between 07.30 and 10.00 h (the pond temperature had reached 26 °C by 10.00 h); the remaining fish were sampled between 16.00 and 18.00 h (at a pond temperature of 34·2 °C and an air temperature of 36·1 °C). Fish were killed on site by decapitation and, following removal of the skin and internal organs, the skeletal muscle (including the spinal cord and ribs) was frozen on dry ice for transport to the laboratory.

Membrane preparation

A crude sarcoplasmic reticulum preparation was obtained as described by Martonosi & Halpern (1972). For each sampling time, frozen tissue from each species was divided into three or four, 7–8 g portions to provide replicate analyses. Freshly thawed tissue was homogenized in 4 vols of imidazole buffer (10 mmol l^{-1}, pH 7·3, containing 0·1 mol l^{-1} KCl) employing a VirTis homogenizer, and the resulting homogenate centrifuged at 3100 *g* for 30 min. The supernatant was next centrifuged at 100 000 *g* for 60 min and the crude membrane pellet resuspended in 0·5 ml of homogenizing buffer prior to being layered on top of a sucrose gradient running from 0·25 to 2 mol l^{-1}. Sucrose gradients were centrifuged for 2 h at 30 000 revs min^{-1} in a SW41 rotor, resulting in the resolution of two major bands which were collected in homogenizing buffer and frozen for subsequent analysis.

Lipid extraction and analyses

Membrane lipids were extracted according to the procedure of Bligh & Dyer (1959) and phospholipid (which remained at the origin) was resolved from other lipids by thin layer chromatography (500 μm plates of silica gel G) in a solvent system of hexane/diethylether/formate (80:20:2) (Christie, 1982). The phospholipid composition was subsequently determined by Iatroscan TLC/FID, as previously described (Hazel, 1985). Individual phospholipid classes were resolved by isocratic elution of a normal phase silica HPLC column (Altex, Ultrasphere-Si, 5 μm, 4·6 mm × 15 cm) with hexane/2-propanol/25 mmol l^{-1} phosphate buffer/[illegible]anol/acetic acid (367:490:62:100:0·6) according to the method of Patton *et al.* (1982). Molecular species compositions were determined by gas chromatography

(SP-2330 column, 10 m, isothermal at 248°C, Hewlett Packard 5840A chromatograph). Trimethylsilyl derivatives of diacylglycerols prepared from native phospholipids [by digestion with phospholipase C according to a modification of the methods of Myher & Kuksis (1982) as previously reported (Hazel & Zerba, 1986)] were analyzed to yield molecular species compositions. Identification of species was accomplished primarily by reference to published retention times (Myher & Kuksis, 1982; Hazel & Zerba, 1986) and by independent analysis of fatty acid compositions by gas chromatography of fatty acid methyl esters (Christie, 1982). Molecular species determinations *via* gas chromatography alone do not permit the assignment of specific fatty acids to the sn-1 or sn-2 position of a phospholipid. In the tables of molecular species composition, the saturated (or less unsaturated) fatty acid of a pair is arbitrarily listed first.

Enzyme assays and analytical procedures

Calcium ATPase, 5′-nucleotidase and glucose-6-phosphatase were assayed by measuring the liberation of inorganic phosphate employing the assay media defined by Moore *et al.* (1981) and Aronson & Touster (1974), respectively; inorganic phosphate was measured according to Chen *et al.* (1957). Succinate– and NADPH–cytochrome *c* reductase activities were estimated by monitoring the reduction of cytochrome *c* at 550 nm, as described by King (1967). Catalase activity was determined by monitoring the reduction in absorbance at 240 nm (due to peroxide), as described by Beers & Sizer (1952). Protein was determined according to the procedure of Bradford (1976), employing bovine serum albumin as a standard.

Statistical analyses

Differences between the mean values for the morning and afternoon sampling periods within a species were tested for statistical significance by means of Student's *t*-test, following arcsine transformation of the percentage data (Sokal & Rohlf, 1981). Use of the word 'significant' in the text indicates a probability of less than 0·05.

Materials

Substrates for enzymatic assays, phospholipase C (*Clostridium welchii*, type I, P-7633) and phospholipid molecular species standards were from Sigma Chemical Co. Sylon HTP and the fused silica capillary column of SP-2330 were from Supelco, Inc. The Ultrasphere-Si, Altex HPLC column was supplied by Beckman Instruments. Organic solvents were washed free of contaminants, if necessary (Kates, 1986), and redistilled prior to use. All solvents employed in the chromatography of phospholipids were of HPLC grade.

Results

Characterization of membrane fractions

The activity of Ca^{2+}-ATPase was enriched two- to threefold (relative to the

Table 1. *The recovery of marker enzyme activities in membrane fractions*

Species Enzyme	Membrane fraction Sarcoplasmic reticulum	 Microsomes
Agosia		
Calcium ATPase	1·73 ± 0·05 (7)*	0·61 ± 0·05 (7)
Glucose-6-phosphatase	ND	0·69 ± 0·09 (7)
5′-Nucleotidase	0·17 ± 0·03 (7)	0·76 ± 0·08 (7)
Succinate–cytochrome *c* reductase	ND	1·04 ± 0·27 (7)
NADPH–cytochrome *c* reductase	0·37 ± 0·20 (2)	2·72 ± 0·34 (7)
Catalase	0·38 ± 0·04 (2)	0·07 ± 0·01 (5)
Notropis		
Calcium ATPase	2·89 ± 0·38 (8)	0·57 ± 0·09 (8)
Glucose-6-phosphatase	ND	0·78 ± 0·16 (8)
5′-Nucleotidase	0·62 ± 0·05 (8)	1·44 ± 0·16 (6)
Succinate–cytochrome *c* reductase	ND	0·45 ± 0·33 (6)
NADPH–cytochrome *c* reductase	ND	5·68 ± 1·87 (6)
Catalase	ND	0·22 ± 0·14 (4)

* Results expressed as relative specific activities (% of total enzyme activity recovered in the membrane fraction/% of total cell protein recovered in the membrane fraction); presented as mean ± S.E.M. for (*N*) preparations.

ND, no activity detected.

crude homogenate) in the membrane fraction designated as sarcoplasmic reticulum, whereas other marker enzymes were either not detectable or three- to fivefold less active in this fraction than in the crude homogenate (Table 1). This sarcoplasmic reticular fraction comprised 7–8 % of the total muscle protein and exhibited levels of Ca^{2+}-ATPase activity (2–3 μmol min^{-1} mg^{-1}) comparable to those reported for sarcoplasmic reticulum isolated from rabbit skeletal muscle (Chu *et al.* 1988). The other prominent membrane band contained low levels of Ca^{2+}-ATPase, but was enriched (three- to sixfold in *Agosia* and *Notropis*, respectively) with NADPH–cytochrome *c* reductase and, in the case of red shiner, moderately enriched (1·4-fold) with 5′-nucleotidase. Correspondingly, this fraction has been designated as 'microsomes' and is presumed to consist of a mixture of smooth endoplasmic reticulum [based on the enrichment with NADPH–cytochrome *c* reductase and the low recovery of glucose-6-phosphatase (Fleischer & Kervina, 1974)] and, at least in the case of red shiner, plasma membranes. The microsomal fraction thus defined accounted, on average, for approximately 2 % of the total cell protein.

Phospholipid composition

The phospholipid composition of the sarcoplasmic reticular fraction did not vary significantly with sampling time (temperature) in either species (Table 2). In contrast, the proportions of phospholipids in the microsomal fraction changed

Table 2. *The phospholipid compositions of sarcoplasmic reticular and microsomal membranes of* Agosia chrysogaster *and* Notropis lutrensis

Membrane / Phospholipid	*Agosia* a.m. (21°C)	*Agosia* p.m. (34°C)	*Notropis* a.m. (26°C)	*Notropis* p.m. (34°C)
Sarcoplasmic reticulum				
Phosphatidylethanolamine	16·55 ± 1·89	17·28 ± 3·19	21·70 ± 1·09	23·47 ± 0·90
Phosphatidylcholine	79·10 ± 2·25	78·91 ± 3·75	73·70 ± 1·30	72·00 ± 0·45
Sphingomyelin	3·82 ± 1·06	3·51 ± 0·71	4·15 ± 0·56	5·37 ± 0·67
Microsomes				
Phosphatidylethanolamine	12·10 ± 2·47	1·54 ± 0·47*	9·70 ± 5·72	11·10 ± 1·18
Phosphatidylcholine	71·30 ± 2·44	82·40 ± 1·95*	78·50 ± 0·33	75·70 ± 1·30
Sphingomyelin	15·06 ± 3·37	11·78 ± 2·80	10·20 ± 0·77	10·00 ± 0·41
Phosphatidylinositol	0·44 ± 0·16	2·12 ± 0·23*	1·06 ± 0·14	5·00 ± 0·94*
Cardiolipin	0·89 ± 0·16	1·51 ± 0·31	0·30 ± 0·04	2·70 ± 0·37*

In addition to the phospholipids listed, phosphatidylserine, phosphatidylinositol and cardiolipin were also present in small (<1 %) quantities in sarcoplasmic reticular membranes.

Composition is expressed as a percentage of total phospholipid by mass and is presented as mean ± S.E.M. for three preparations.

* Denotes a significant difference ($P < 0·05$) between morning and afternoon samples.

significantly between morning and afternoon samples, but in a species-specific manner. In *Agosia*, the proportion of PE decreased significantly while the proportion of PC increased between the cool of the morning and the heat of the afternoon. Consequently, the ratio of PC/PE increased nearly 10-fold during the day from 5·9 in the morning to 53·5 in the afternoon. Similar to the response of PC, levels of PI were significantly higher in fish sampled in the afternoon. In contrast, proportions of the major phospholipids (PC and PE) did not vary significantly with sampling time in *Notropis*. However, levels of both PI and cardiolipin were significantly elevated in fish collected in the heat of the afternoon as opposed to early morning.

Phospholipid molecular species compositions

Generally speaking, the molecular species compositions of neither PC (Table 3, Fig. 1, top) nor PE (Table 4., Fig. 1, bottom) varied with sampling time in the sarcoplasmic reticulum of either fish species. No statistically significant differences were established between morning and afternoon samples for the molecular species compositions of PE in either species, or for species of PC in *Agosia*. However, in both *Agosia* and *Notropis*, unsaturated/saturated (U/S) ratios for PC were slightly higher in the morning samples (7·89 *vs* 6·64 for *Agosia* and 4·74 *vs* 2·54 for *Notropis*), due largely, in *Agosia,* to higher proportions (30·36 *vs* 22·3 %) of dienoic molecular species (16:1/18:1, 18:1/18:1 and 18:0/18:2) and, in *Notropis*, to higher levels of pentaenoic species (29·6 *vs* 7·8 %, primarily 18:0/20:5 and 16:0/20:5). Diunsaturated species of PC (those species possessing

Table 3. *Molecular species profiles of phosphatidylcholine in membranes of* Agosia chrysogaster *and* Notropis lutrensis

Species	Sarcoplasmic reticulum		Microsomal membranes	
	a.m. (21°C) ($N = 4$)	p.m. (34°C) ($N = 3$)	a.m. (21°C)	p.m. (34°C)
Agosia				
30[0,0]	5·61 ± 2·51	4·20 ± 1·24	7·27	52·46
30[0,1]	0·53 ± 0·53	0·02 ± 0·02	22·54	15·34
30[0,2]	0·38 ± 0·38	1·33 ± 1·08	3·72	3·76
32[0,0]	7·29 ± 2·49	8·88 ± 0·13	10·68	4·96
34[0,1]	19·92 ± 4·78	23·22 ± 3·46	37·53	13·36
34[1,1]	11·60 ± 5·03	5·48 ± 3·29	–	–
36[0,1]	3·61 ± 0·29	2·28 ± 0·63	4·80	4·72
36[0,2]	6·26 ± 1·20	0·37 ± 1·23	–	–
36[0,4]	14·81 ± 5·01	15·08 ± 7·58	4·24	2·95
36[1,1]	11·88 ± 4·81	7·85 ± 6·36	–	–
38[0,6]	16·39 ± 1·54	19·60 ± 2·57	7·23	2·46
Notropis				
	a.m. (26°C) ($N = 1$)	p.m. (34°C) ($N = 4$)	a.m. (26°C)	p.m. (34°C)
30[0,0]	11·31	22·66 ± 1·87	7·44	38·38
30[0,1]	5·80	22·27 ± 4·79	12·06	29·79
30[0,2]	2·77	3·93 ± 0·46	2·74	3·51
32[0,0]	6·08	6·99 ± 1·34	3·86	0·29
32[0,2]	–	–	2·07	–
34[0,1]	19·08	11·08 ± 1·25	15·25	3·74
34[1,1]	3·99	4·02 ± 0·65	2·20	0·70
36[0,1]	1·59	2·24 ± 1·18	6·59	1·54
36[0,2]	3·31	3·87 ± 0·57	3·42	1·69
36[0,4]	–	–	4·02	1·28
36[0,5]	19·22	7·79 ± 1·13	–	–
36[1,1]	7·74	0·91 ± 0·91	–	–
36[2,4]	2·34	4·47 ± 0·81	–	–
38[0,4]	1·50	1·01 ± 0·59	10·23	4·49
38[0,5]	10·36	–	2·64	5·06
38[0,6]	–	6·79 ± 2·15	8·61	5·30
38[2,4]	3·84	–	8·81	3·23
38[1,6]	–	–	5·38	–

Terminology: The first number represents the combined length (number of carbon atoms) of the acyl groups esterified to the 1- and 2- positions of the phospholipid glycerol backbone; the numbers in brackets refer to the number of double bonds in each of the acyl chains, e.g. 1–16:0, 2–22:6 PC is designated as 38[0,6]. Molecular species analysis does not permit the assignment of a particular fatty acid to a specific position on the glycerol backbone. In the notation adopted for this table, the saturated (or less unsaturated) fatty acid of a pair is arbitrarily listed first; this does not necessarily mean that this fatty acid is located at the 1-position. Molecular species assignments are: 30[0,0], 14:0/16:0; 30[0,1], 14:0/16:1; 30[0,2], 14:0/16:2; 32[0,0], 16:0/16:0; 32[0,2], 16:0/16:2; 34[0,1], 16:0/18:1; 34[1,1], 16:1/18:1; 36[0,1], 18:0/18:1; 36[0,2], 18:0/18:2; 36[1,1], 18:1/18:1; 36[0,4], 16:0/20:4; 36[0,5], 16:0/20:5; 36[2,4], 16:2/20:4; 38[0,4], 18:0/20:4; 38[0,6], 16:0/22:6; 38[2,4], 18:2/20:4; 38[0,5], 18:0/20:5; 38[1,6], 16:1/22:6.

Only molecular species comprising 2% or more of the composition for at least one of the experimental groups indicated are reported.

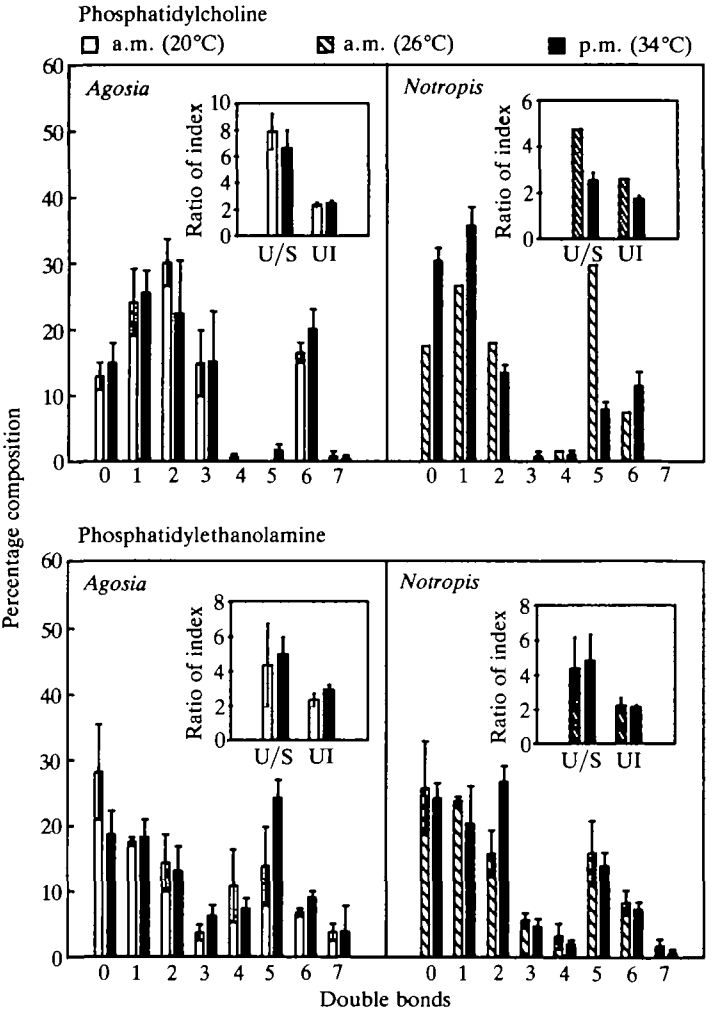

Fig. 1. The molecular species compositions of phosphatidylcholine and phosphatidylethanolamine in sarcoplasmic reticular membranes of *Agosia chrysogaster* and *Notropis lutrensis* sampled in the early morning (a.m. at 21°C for *Agosia* and 26°C for *Notropis*) and late afternoon (p.m. at 34°C) grouped according to degree of unsaturation. U/S, ratio of unsaturated to saturated molecular species; UI, unsaturation index (the average number of double bonds per molecular species). Values are means ± S.E.M. (For values of *N*, see Tables 3 and 4.)

an unsaturated fatty acid at both the sn-1 and sn-2 positions) predominated in the morning samples of both fish species (41·4 *vs* 33·8 % for *Agosia* and 28·3 *vs* 16·2 % for *Notropis*). In contrast, U/S ratios and the proportions of diunsaturated molecular species displayed opposite tendencies in PE, being, if anything, slightly greater in the afternoon as opposed to morning samples.

Variations in the phospholipid molecular species composition of the microsomal

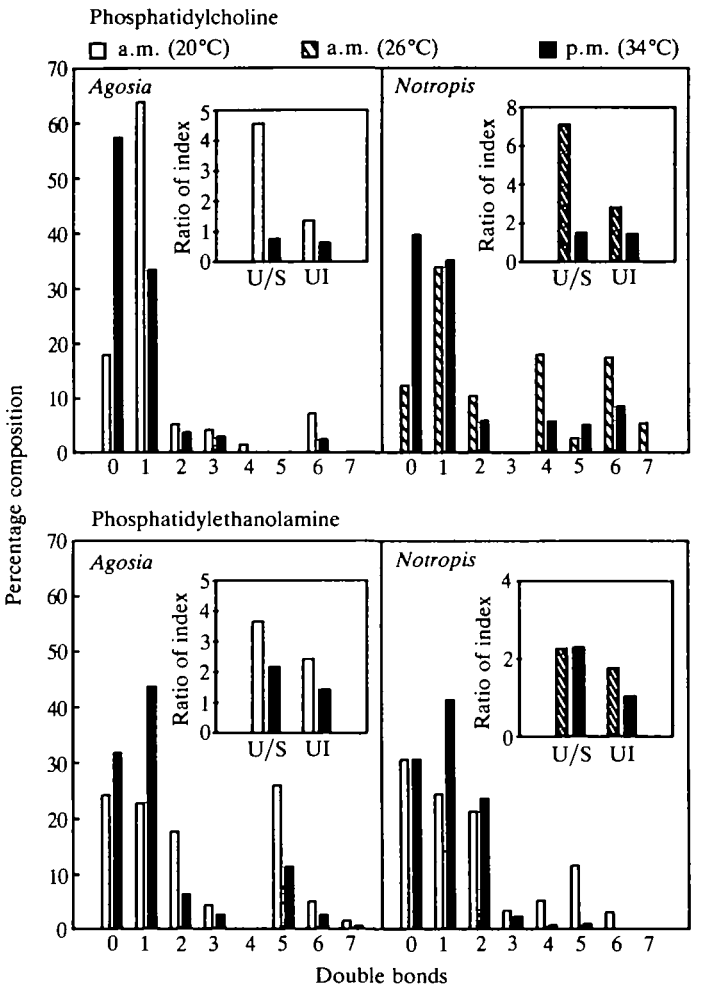

Fig. 2. The molecular species compositions of phosphatidylcholine and phosphatidylethanolamine in microsomal membranes of *Agosia chrysogaster* and *Notropis lutrensis* sampled in the early morning (a.m. at 21°C for *Agosia* and 26°C for *Notropis*) and late afternoon (p.m. at 34°C) grouped according to degree of unsaturation. U/S, ratio of unsaturated to saturated molecular species; UI, unsaturation index (the average number of double bonds per molecular species).

fraction with sampling time, although not subject to statistical analysis (to ensure adequate material for study, the microsomal fractions from all preparations at a given sampling time were pooled), were consistently more evident in the microsomal than the sarcoplasmic reticular fraction (Fig. 2 and Tables 3 and 4). For PC, both the U/S ratio and unsaturation index (UI) were substantially greater in morning than afternoon samples (7·48 *vs* 0·77 and 7·1 *vs* 1·52 for the U/S ratios of *Agosia* and *Notropis*, respectively; 1·36 *vs* 0·65 and 2·82 *vs* 1·46 for UI); hexaenoic (16:0/22:6) species contributed to the increased unsaturation in both

fish species, while monoenoic (63·9 *vs* 33·4 %, primarily 14:0/16:1 and 16:0/18:1) and tetraenoic species (17·98 *vs* 5·77 %, primarily 16:0- and 18:0/20:4) also contributed in *Agosia* and *Notropis*, respectively. Diunsaturated species of PC were more abundant in the morning than afternoon samples for both teleost species (7·23 *vs* 2·46 % in *Agosia* and 29·48 *vs* 14·28 % in *Notropis*). Similar but less striking patterns are also apparent in the molecular species composition of PE. In microsomal membranes of *Agosia*, both the U/S ratio (3·66 *vs* 2·17) and unsaturation index (2·43 *vs* 1·43) were higher in morning than afternoon samples, reflecting increased proportions of dienoic (17·7 *vs* 6·47 %, primarily 14:0/16:2 and 16:0/16:2), pentaenoic (26 *vs* 11·5 %, primarily 18:1/20:4) and hexaenoic species and reduced amounts (22·42 *vs* 31·78 %) of saturated species. In *Notropis*, the ratio of unsaturated to saturated species of PE remained constant, but the unsaturation index was greater in morning samples compared to those taken later in the day, owing to a reduced abundance of monoenoic species (from 41·3 *vs* 24·4 %, primarily 14:0/16:1) and increased proportions of tetra-, penta- (from 1·06 to 11·64 %, 18:0/20:5 and 16:0/20:5) and hexaenoic species. Similar to the

Table 4. *Molecular species profiles of phosphatidylethanolamine in membranes of* Agosia chrysogaster *and* Notropis lutrensis

	Sarcoplasmic reticulum		Microsomal membranes	
Species	a.m. (21 °C) ($N = 4$)	p.m. (34 °C) ($N = 3$)	a.m. (21 °C)	p.m. (34 °C)
Agosia				
30[0,0]	17·28 ± 5·41	13·72 ± 4·61	18·37	28·73
30[0,1]	–	–	5·64	12·04
30[0,2]	5·93 ± 3·33	0·44 ± 0·44	11·56	5·20
32[0,0]	2·15 ± 1·26	–	–	–
32[0,1]	4·19 ± 1·77	3·48 ± 0·60	5·87	17·85
32[0,2]	2·87 ± 1·07	0·94 ± 0·94	3·48	–
34[0,0]	8·33 ± 3·48	1·37 ± 1·37	–	–
34[0,1]	–	–	8·25	11·76
34[0,2]	–	–	–	1·27
36[0,0]	0·44 ± 0·44	3·60 ± 3·60	–	3·05
36[0,1]	13·35 ± 2·34	14·79 ± 1·25	3·05	2·02
36[0,2]	4·59 ± 1·07	8·13 ± 1·27	1·07	–
36[0,3]	3·73 ± 1·20	6·27 ± 1·59	4·42	2·71
36[1,1]	–	2·29 ± 1·70	–	–
36[2,4]	2·84 ± 2·17	4·08 ± 1·97	–	1·05
38[0,4]	10·30 ± 5·49	7·34 ± 0·94	–	–
38[0,5]	3·40 ± 1·24	3·91 ± 3·91	–	–
38[1,4]	8·31 ± 5·04	15·30 ± 1·12	24·49	10·30
38[2,4]	1·23 ± 0·74	–	3·64	1·62
38[2,5]	4·65 ± 2·65	3·04 ± 1·82	4·04	0·58
40[0,5]	2·08 ± 1·29	4·70 ± 2·37	–	–
40[0,6]	2·69 ± 0·65	4·94 ± 0·98	1·47	–

Table 4. *Continued*

Species	Sarcoplasmic reticulum		Microsomal membranes	
	a.m. (26°C) ($N = 3$)	p.m. (34°C) ($N = 4$)	a.m. (26°C)	p.m. (34°C)
Notropis				
30[0,0]	18·54 ± 3·83	24·25 ± 2·25	30·39	29·09
30[0,1]	14·20 ± 1·71	11·63 ± 3·15	5·94	26·24
30[0,2]	3·59 ± 3·59	10·99 ± 2·65	12·61	10·06
32[0,0]	7·18 ± 3·63	–	–	–
32[0,1]	2·54 ± 1·63	4·24 ± 1·28	9·66	8·51
32[0,2]	2·44 ± 1·30	2·82 ± 1·37	4·38	7·31
34[0,1]	7·08 ± 0·69	4·51 ± 1·72	4·65	3·85
34[0,2]	0·73 ± 0·73	–	–	3·12
34[1,1]	–	–	1·57	2·15
34[1,2]	–	–	3·48	2·42
36[0,1]	–	–	4·19	2·72
36[0,2]	3·24 ± 0·39	4·58 ± 0·98	–	–
36[0,3]	5·61 ± 1·10	4·64 ± 1·19	–	–
36[0,5]	–	–	5·45	1·06
36[1,1]	5·71 ± 1·16	3·23 ± 3·23	2·85	1·04
36[2,2]	–	–	2·24	0·84
36[2,4]	3·34 ± 0·88	5·71 ± 2·15	3·16	–
38[0,4]	3·21 ± 1·93	2·02 ± 1·17	3·09	–
38[0,5]	–	–	6·19	–
38[1,4]	14·33 ± 3·30	10·03 ± 0·81	–	–
40[0,5]	1·11 ± 1·11	3·87 ± 0·92	–	–
40[0,6]	4·93 ± 1·88	1·59 ± 1·08	–	–

Terminology and molecular species assignments are as defined in Table 3, with the following additions/changes: 32[0,1], 16:0/16:1; 34[0,0], 16:0/18:0; 34[0,2], 16:0/18:2; 36[0,0], 18:0/18:0; 36[0,1], 18:0/18:1; 36[0,2], 18:0/18:2; 36[0,3], 18:0/18:3; 36[2,2], 18:2/18:2; 38[0,5], 18:0/20:5; 38[1,4], 18:1/20:4; 38[2,5], 18:2/20:5; 40[0,5], 18:0/22:5; 40[0,6], 18:0/22:6.

Only molecular species comprising 2% or more of the composition for one of the experimental conditions indicated are reported.

pattern in PC, diunsaturated species of PE were more abundant in morning than afternoon samples (35·3 *vs* 14·21% in *Agosia* and 19·48 *vs* 6·45% in *Notropis*).

Only microsomal membranes from *Notropis* yielded sufficient phosphatidylinositol (PI) for molecular species analysis (Table 5, Fig. 3). Although total unsaturation (measured either as U/S ratio or UI) did not vary significantly with sampling time, morning samples contained significantly greater proportions of monoenoic (47·1 *vs* 33·3%, due primarily to 14:0/16:1) and lower proportions of di- and trienoic species.

Discussion

The present experiments clearly demonstrate that biological membranes can

Table 5. *Molecular species profile of phosphatidylinositol in plasma membranes of* Notropis lutrensis

Species	a.m. (26°C) ($N = 3$)	p.m. (34°C) ($N = 3$)
30[0,0]	11·07 ± 1·99	6·18 ± 3·58
30[0,1]	39·95 ± 5·29	24·47 ± 6·10
30[0,2]	2·89 ± 1·96	9·29 ± 3·95
32[0,0]	1·89 ± 1·89	4·42 ± 0·97
32[0,1]	3·37 ± 1·43	2·36 ± 0·55
32[0,2]	0·99 ± 0·99	3·06 ± 1·57
34[0,1]	2·66 ± 1·44	1·89 ± 0·19
34[0,3]	1·76 ± 0·96	3·89 ± 2·43
34[1,1]	2·22 ± 1·17	2·24 ± 1·91
36[0,0]	1·59 ± 1·00	3·14 ± 1·57
36[0,1]	1·13 ± 1·13	5·06 ± 1·66
36[0,2]	0·52 ± 0·52	2·04 ± 2·04
36[0,3]	–	5·99 ± 3·00
36[0,5]	5·27 ± 2·17	0·98 ± 0·98
36[1,1]	1·91 ± 0·30	5·12 ± 2·21
36[2,2]	4·81 ± 4·03	4·07 ± 0·93
36[2,3]	3·00 ± 3·00	5·05 ± 2·54
38[0,4]	5·22 ± 0·75	4·18 ± 0·49
38[0,5]	1·48 ± 0·74	2·22 ± 1·14
38[1,4]	1·82 ± 1·82	1·72 ± 1·72
38[1,6]	3·24 ± 0·42	1·06 ± 0·29*
38[2,4]	1·80 ± 0·93	1·22 ± 1·22
38[2,5]	0·61 ± 0·61	1·34 ± 1·34
40[0,5]	0·67 ± 0·67	0·68 ± 0·68
40[0,6]	0·16 ± 0·16	–

Terminology and molecular species assignments are as defined in Table 3, with the following additions/changes: 32[0,1], 16:0/16:1; 34[0,3], 16:0/18:3; 36[0,0], 18:0/18:0; 36[0,3], 18:0/18:3; 36[2,2], 18:2/18:2; 36[2,3], 18:2/18:3; 38[0,5], 18:0/20:5; 38[1,4], 18:1/20:4; 38[2,4], 18:2/20:4; 38[2,5], 18:2/20:5; 40[0,5], 18:0/22:5; 40[0,6], 18:0/22:6.

Only molecular species comprising 2% or more of the composition for one of the experimental conditions indicated are reported.

* Means are significantly ($P < 0·05$) different.

undergo dramatic changes in lipid composition within the course of an 11- to 12-h period. The temporal restructuring of lipid composition in muscle cell membranes was more evident in the microsomal than the sarcoplasmic reticular fraction and was most consistently manifest in the altered molecular species composition of phosphatidylcholine. Although compositional adjustments were to some extent species- and phospholipid-specific, common patterns emerge. In microsomal membranes, the ratio of unsaturated to saturated molecular species of PC was five- to 10-fold higher in morning than afternoon samples, reflecting substantial reductions in the abundance of saturated molecular species and corresponding increased proportions of unsaturated ones. The degree of molecular species

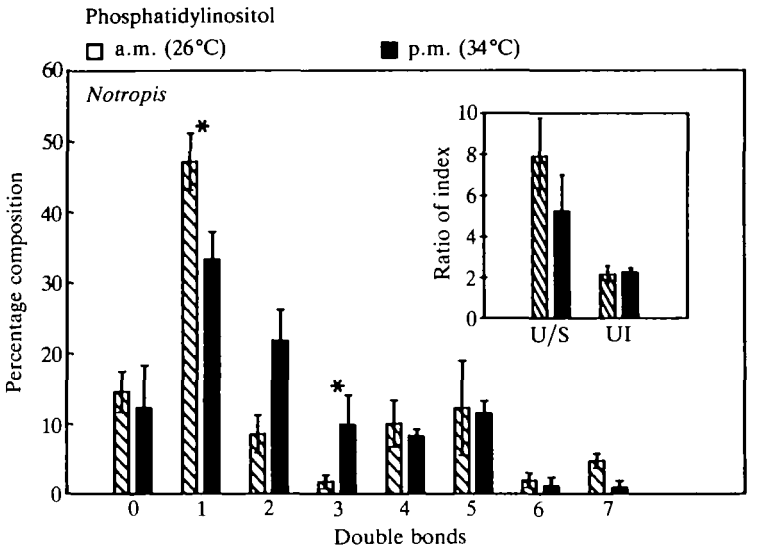

Fig. 3. The molecular species composition of phosphatidylinositol in microsomal membranes of *Notropis lutrensis* sampled in the early morning (a.m. at 26°C) and late afternoon (p.m. at 34°C) grouped according to degree of unsaturation. U/S, ratio of unsaturated/saturated molecular species; UI, unsaturation index (the average number of double bonds per molecular species). * Denotes a significant difference ($P < 0{\cdot}05$) between the morning and afternoon samples. Values are means ± S.E.M. ($N = 3$).

unsaturation (as indicated by changes in UI) was also higher in morning than afternoon samples, but less dramatically (twofold) so. Qualitatively similar but smaller adjustments were also evident in microsomal PE and, although not significant, in addition, the proportions of diunsaturated molecular species of both PC and PE were consistently two- to threefold higher in morning than afternoon samples. Conversely, neither the unsaturation ratio nor the unsaturation index varied significantly with time of day in microsomal phosphatidylinositol. However, monoenoic molecular species were more abundant in the morning than afternoon hours.

Changes in phospholipid headgroup composition were neither as striking nor as widespread as those in molecular species composition, but were again largely restricted to the microsomal fraction. Furthermore, only in *Agosia* were proportions of the major phosphatides (PC and PE) altered significantly, as shown by a 10-fold rise in the PC/PE ratio between the morning and afternoon sampling periods. Conversely, proportions of PI increased significantly in microsomes of both fish species over the same period.

The variations in membrane lipid composition reported in this study, being manifest in a 7- to 11-h period, are among the fastest recognized adjustments to environmental change. Proportions of PE in microsomal membranes of *Agosia* declined more rapidly in response to diurnal warming than similar changes in plasma membranes of trout kidney during acclimation from 5 to 20°C, in which the

PE/PC ratio did not decline significantly for 2 days (Hazel & Landrey, 1988*a*). Rates of change in molecular species composition, however, were comparable to those previously reported in plasma membranes of trout kidney, for which proportions of monoenoic species of PC declined significantly within 8 h of warm acclimation (from 5 to 20°C) and rose to a comparable extent between 24 and 48 h of cold acclimation; similarly, proportions of saturated species had decreased significantly by hour 16 of the cold acclimation (Hazel & Landrey, 1988*b*).

It is not possible, based on results of the present experiments, to determine whether the observed changes in membrane lipid composition represent, in actuality, a response to altered temperature, or a diurnal pattern regulated in response to other environmental cues. Although we would have liked to maintain a control group at constant temperature during the period of daily warming, reproducing variable attributes (other than temperature) of the natural environment under laboratory conditions is difficult, making any extrapolation to the field situation tenuous. Nevertheless, it would be of particular interest to determine whether changes in lipid composition precede, and thus anticipate, extremes of diurnal temperature fluctuation or persist in the absence of cycling temperatures. Regardless of the regulatory mechanism, the similarity between the present results and temperature-induced alterations in membrane lipid composition suggests that some degree of thermal compensation in membrane function (i.e. homeoviscous adaptation) may occur as a consequence of these adjustments. Depressed values for both the ratio of unsaturated to saturated molecular species and the unsaturation index of microsomal PC are characteristic of desert fish captured in the heat of the afternoon (Fig. 2), and are quite similar to the patterns reported for membranes of trout liver (Hazel & Zerba, 1986) and kidney (Hazel & Landrey, 1988*b*) following a period of warm acclimation (to 20°C). The markedly reduced proportions of diunsaturated molecular species in both PC and PE in fish sampled in the heat of the afternoon are, however, a unique feature of the present study. Diminished proportions of the highly fluid, diunsaturated molecular species of phospholipids may be a necessity in offsetting the fluidizing effect of elevated temperature upon membrane order (Lynch & Thompson, 1984). Similarly, elevated proportions of PC, comparable to those observed in microsomal membranes of *Agosia* in late afternoon, are a common feature of adaptation to warm temperature (Hazel, 1988). Furthermore, in a comparison between species of crayfish which differ in overwintering behavior, only species which remain active at the extremes of environmental temperature display altered headgroup compositions (Pruitt, 1988). Such changes in headgroup composition may be important in maintaining an appropriate balance between bilayer(PC)- and nonbilayer(PE)-forming lipids in the membrane (Hazel, 1989).

The observed changes in phospholipid molecular species composition are presumed to arise primarily from deacylation/reacylation cycle activity [i.e. phospholipase-catalyzed cleavage of a fatty acid from either the sn-1 or the sn-2 position of a membrane-resident phospholipid (Neas & Hazel, 1985), followed by reacylation of the resulting lysophosphatide with a different acyl chain to form a

new molecular species (Livermore & Hazel, 1988)], since altered capacities for fatty acid desaturation evolve only slowly in response to temperature change (Hagar & Hazel, 1985). The consistently larger adjustments in the ratio of unsaturated to saturated molecular species and in the proportions of diunsaturated *vs* mixed-chain (i.e. one saturated and one unsaturated chain) species than in the average degree of species unsaturation (UI) in both PC and PE further implicate a greater contribution of acyl chain reshuffling as opposed to acyl chain modification to the observed changes in molecular species composition.

Remarkably, in neither *Agosia* nor *Notropis* were most aspects (including the phospholipid class composition and molecular species composition of PE in both fish, and the molecular species composition of PC in *Agosia*) of the lipid composition of sarcoplasmic reticular membranes significantly influenced by sampling time. This observation is consistent with the results of previous studies documenting that sarcoplasmic reticular membranes exhibit no capacity for homeoviscous adaptation (Cossins *et al.* 1978).

The demonstration of headgroup restructuring in microsomal membranes of *Agosia* but not *Notropis*, coupled with the greater extent of molecular species restructuring in the former, particularly in PE, may indicate that *Agosia* is better able to match the lipid composition of its biological membranes to ambient thermal conditions. However, because *Notropis* were sampled later in the morning (after the sun had risen), the initial sampling temperature was higher and the difference in temperature between sampling intervals was smaller for *Notropis* than *Agosia*. Consequently, membranes of *Notropis* may have experienced a significant change in lipid composition prior to the initial sampling period (as the water temperature warmed from 21 to 26°C), thereby diminishing the magnitude of the changes detected by late afternoon (34°C). Thus, whether observed differences in the magnitude of the temperature-induced changes in membrane lipid composition between *Notropis* and *Agosia* are real or artifactual cannot be resolved by the present data. Furthermore, because *Notropis* has been widely introduced throughout the southwest, it is not possible to determine whether species distributions are correlated with apparent differences in adaptive capacity (Minckley, 1973).

In summary, both the phospholipid molecular species (in both *Agosia* and *Notropis*) and headgroup (in *Agosia* only) compositions of microsomal membranes experience dramatic diurnal fluctuations in two species of Sonoran desert teleosts. The pattern of these variations in relation to the substantial diurnal fluctuations in water temperature characteristic of the desert environment suggests a role for membrane restructuring in the maintenance/stabilization of membrane function. Remodelling of phospholipid molecular species composition *via* membrane-resident enzymes of the deacylation/reacylation cycle appears to be the most common mechanism for rapidly modifying membrane composition in these species. In contrast, the lipid composition of sarcoplasmic reticular membranes is refractory to change, implying the absence of lipid-mediated homeoviscous adaptation in these membranes.

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