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Temperature adaptation of biological membranes: differential homoeoviscous responses in brush-border and basolateral membranes of carp intestinal mucosa

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The effects of temperature acclimation of carp upon the hydrocarbon order of intestinal membranes has been determined. A fractionation technique has been developed for the simultaneous purification of brush-border and basolateral membrane fractions from the intestinal mucosa. The specific activity of alkaline phosphatase in the brush-border fraction was enhanced 6.4-fold over that of the initial homogenate, whilst the (Na^+-K^+)-stimulated ATPase was enhanced 5.8-fold in the basolateral fraction. The specific activities of NADPH–cytochrome-*c* reductase, succinate–cytochrome-*c* reductase and acid phosphatase were not increased in these two fractions. Membrane hydrocarbon order in membranes from 10 and 30 °C-acclimated carp has been compared by measuring the steady-state fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene over a range of temperatures. In the brush-border fraction, polarization was identical in both cold- and warm-acclimated groups, whilst large differences were observed in the basolateral fraction sufficient to offset approx. 75% of the temperature-induced ordering effects of cold. The fatty acid composition of the major phosphoglyceride fractions in the brush-border fraction was also largely unaffected by thermal acclimation, whilst the basolateral fraction showed significant increases in the proportion of unsaturated fatty acids in the cold. It is concluded that whilst the basolateral membrane of intestinal mucosa displays a large homoeoviscous response that correlates with a shift in lipid composition, the brush-border membrane does not. These findings are consistent with evidence of functional adaptations of the basolateral membrane during thermal acclimation (Gibson, J.S., Ellory, J.C. and Cossins, A.R. (1985) J. Exp. Biol. 114, 355–364).

Introduction

The adaptive regulation of intestinal morphology and function is a subject that is gaining increasing attention [1]. One of the most dramatic adaptive responses occurs during the acclimation of fish to altered temperature, and this may serve as a useful model of the adaptive regulation of intestinal function generally. The typical response is compensatory, such that function is adjusted to overcome, either partially or completely, the direct effects of the temperature shift and, thereby, provide some longer-term independence from the effects of seasonal changes in temperature. Thus, Smith [2] has shown almost identical electrical potentials across the intestinal mucosa of goldfish acclimated to temperatures between 8 and 30 °C, when measured at their respective acclimation temperatures. More recently, Gibson et al. [3] showed in carp intestine that the transepithelial short circuit current measured at 10 °C (a measure of epithelial Na^+ transport capacity), was approx. 40% greater in cold-acclimated fish than in warm-acclimated fish. They also presented evidence that the increased transport capacity of cold-acclimated intestine was due to an increased activity of the basolateral Na^+ pump rather than in the Na^+ permeability of the brush border.

Thus, the basolateral membrane appears to be a particularly important site for the adaptive regulation of transepithelial transport during temperature acclimation. There is good evidence that the turnover number of the Na^+/K^+-ATPase in various cell types may be influenced by the order and mobility of the surrounding bilayer lipids [4]. This raises the possibility that adjustments to the motional freedom or 'fluidity' of the membrane bilayer may promote the adjustments of pump activity observed during temperature acclimation. The compensatory adjustment of membrane order by

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changes in lipid composition has been widely observed, especially in fish [5,6]. This phenomenon has been called 'homoeoviscous adaptation', a term which emphasizes the homoeostatic nature of the response [7].

We present here a study of changes in membrane order in the basolateral and brush-border membranes of the intestinal mucosa of temperature-acclimated carp. There is a large literature on the fractionation of luminal and contraluminal membrane fractions of transporting epithelial, although most papers have dealt only with the purification of one fraction (usually the brush border fraction), and none deal with the preparation of basolateral membranes from fish. In order to compare the adaptive responses of the two membrane fractions we have developed a method for the simultaneous preparation which combines their Mg^{2+} precipitation technique at a previously untried stage with centrifugation on a discontinuous sucrose gradient.

Materials and Methods

Materials

Deoxycholic acid (DOC), dithiothreitol (DTT), bovine serum albumin (BSA), glycine buffer solution, di-sodium adenosine triphosphate (Na-ATP), reduced β-nicotinamide adenine dinucleotide phosphate (NADPH), ouabain, *para*-nitrophenyl phosphate and nitrophenyl phosphate were purchased from Sigma (Poole, U.K.). Cytochrome *c* was purchased from Boehringer Biochemicals (Lewes, U.K.). Inorganic compounds, sucrose and Folin-Ciocalteau phenol reagent were purchased from BDH (Poole, U.K.). 1,6-Diphenyl-1,3,5-hexatriene (DPH) was obtained from Molecular Probes (Junction City, OR, U.S.A.).

Fish

Common carp (*Cyprinus carpio*, L., 0.25–0.5 kg) were obtained from a commercial source and held in 500 l aquaria at 10 ± 2 °C or 30 ± 0.5 °C, with a 8L : 16D and 16L : 8D photoperiod, respectively, for at least 4 weeks. Animals used for the development of the membrane fractionation procedure were kept at 17 ± 2 °C. Animals were fed to satiation either daily (30 °C) or once every 2 days (10 °C) using trout pellets (BP Nutrition, Northwich, U.K.).

Membrane fractionation procedure (see Fig. 1)

Carp intestines were removed from the animal and the luminal contents were flushed out with ice-cold carp saline containing 115 mM NaCl, 5 mM KCl, 5 mM glucose, 15 mM imidazole (pH 7.68 at room temperature), 2 mM $CaCl_2$. One, or occasionally two, intestines per preparation were everted by rolling the intestine over a clean glass rod inserted along its length. The anterior two-thirds of the intestine was ligated at both ends and was thoroughly washed in carp saline. A large

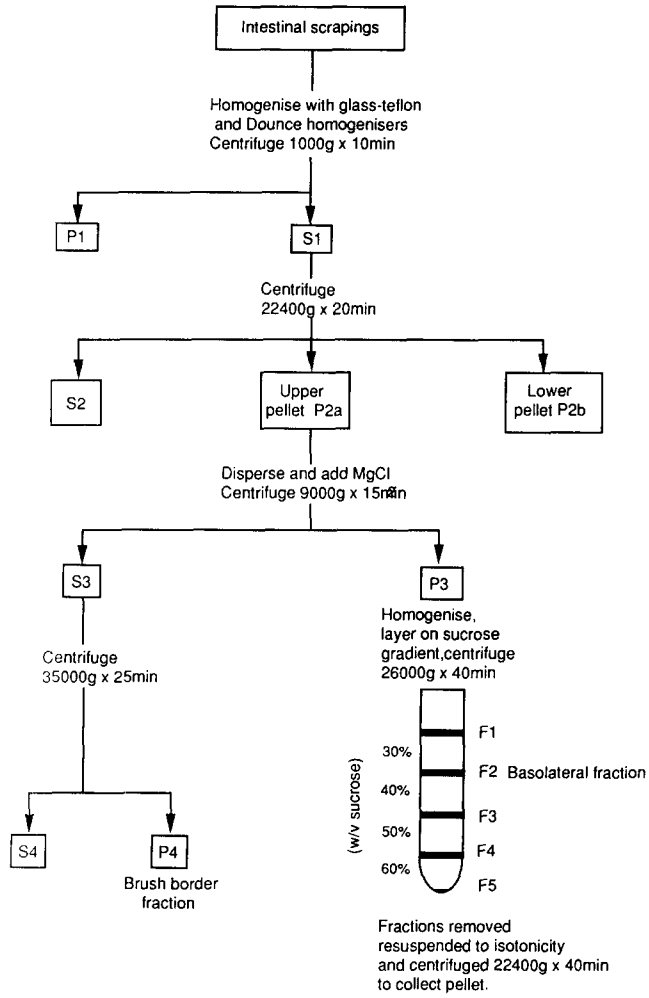

Fig. 1. A simplified flow-diagram of the fractionation procedure for carp intestinal membranes (see Materials and Methods for full details).

proportion of the surface mucus was removed by blotting with absorbent paper and by including 1 mM DTT in the carp saline [8]. The intestine was transferred to ice-cold isolation medium (250 mM sucrose, 5 mM imidazole (pH 7.68)) and the mucosa was gently scraped with a glass coverslip. The remaining tissue was discarded and the solution containing the mucosal scrapings made up to 70 ml with isolation medium. The scrapings were homogenized using 20 passes with a motor-driven glass-Teflon homogeniser (800 rpm), followed by ten passes with a hand-held 'Dounce' type glass-glass homogeniser (Kontes, tight-fitting pestle). The homogenate was centrifuged at 1000 × *g* for 10 min in a Coolspin centrifuge (Fisons MSE, U.K.) with the brake off. Supernatant S1 was removed and centrifuged at 22 400 × *g* for 20 min in a Beckman J-21 centrifuge to yield supernatant S2 and a double-layered pellet. The upper fluffy white part (P2a) was gently washed from the more tightly packed lower pellet (P2b) in a minimal volume of isolation medium and diluted to 10 ml. $MgCl_2$ (1 M solution) was added to this suspension to give a final concentration of 10 mM and after standing

for 20 min, the suspension was centrifuged at $9000 \times g$ for 15 min to give supernatant S3 and pellet P3. Centrifugation of S3 at $35000 \times g$ for 25 min yielded pellet P4 which was enriched in brush-border membranes. Pellet P3 was gently homogenized in a small volume of isolation medium by 3–4 passes of the Dounce-type homogeniser and was layered onto a freshly prepared discontinuous density gradient composed of 5 ml layers of 30, 40, 50 and 60% (w/v) sucrose in 5 mM imidazole (pH 7.68 at room temperature). The gradient was centrifuged in a swing-out rotor at $26000 \times g$ for 40 min (Superspeed 65 centrifuge, MSE with the brake off). Fractions F1–F4 were removed from the interfaces of the sucrose layers using glass Pasteur pipettes. F1 was designated as the material that did not migrate into the gradient and remained at the top, whilst F4 was at the 50–60% interface. No material was observed at the bottom of the tube (F5). Each of these fractions were diluted with distilled water to approx. 300 mosmol $\cdot$ kg^{-1} and recovered by centrifugation at $22400 \times g$ for 45 min.

Protein determination

The content of microsomal protein in each fraction was determined after the method of Lowry et al. [9] as modified by Albro [10] for calcium-precipitated microsomes. The protein measurements were made from samples which had been frozen within 48 h of isolation.

Marker enzyme assays

Alkaline and acid phosphatases. These were used as markers for the brush-border and lysosomal membranes, respectively [11]. For the alkaline phosphatase, membrane aliquots were incubated in 0.5 ml glycine buffer (pH 10) and 0.5 ml of 0.5 mM deoxycholate at room temperature. The reaction was started by adding 0.5 ml 2 mM *p*-nitrophenyl phosphate in glycine buffer and stopped by adding 3 ml of 2 M NaOH. The absorbance was measured at 410 nm. Acid phosphatase was determined in the same manner except that the glycine buffer was replaced by 50 mM sodium acetate buffer adjusted to pH 5 with NaOH. Potassium fluoride (1 mM, final) or EDTA (4 mM, final) were routinely added to the incubation mixtures for the alkaline and acid phosphatase assays, respectively [12].

(Na^+-K^+)-stimulated ATPase. This was used as a marker for basolateral membranes [13]. Membrane aliquots were incubated in a saline composed of 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 0.5 mM DOC and 15 mM imidazole (pH 7.6 at room temperature) either in the presence or absence of ouabain (1 mM final concentration). Sodium azide was routinely included at a final concentration of 2 mM to inhibit mitochondrial ATPase activity. The reaction was initiated by adding 0.5 ml of 15 mM Na-ATP. The reaction was stopped and the inorganic phosphate determined using the lubrol technique [14]. Ouabain-sensitive activity was calculated by subtracting the activity in the presence of ouabain from that in its absence.

NADPH / succinate cytochrome-c reductase. Microsomal and mitochondrial contamination was assessed using the marker enzymes NADPH– and succinate–cytochrome-*c* reductase, respectively, as described by Parkes and Thompson [15]. The reaction rate was calculated using the extinction coefficient for cytochrome *c* of 19.7 $mM^{-1} \cdot cm^{-1}$ [16].

Steady state polarization measurements

The polarization of DPH under steady illumination was measured on the analogue T-format fluorimeter described previously [17,18]. The performance of the fluorimeter was routinely checked using a suspension of glycogen in water (polarization at 500 nm = 0.97–0.98) and diethylnaphthalene sulphonate in water (polarization = 0.002). Cuvette contents were continuously mixed with a magnetic follower driven by an electromagnet (Rank Bros, Bottisham, Cambridge, U.K.) positioned beneath the cuvette. The temperature of the cuvette contents were monitored by a linear thermistor and the results were recorded to floppy disc by the microcomputer. The microcomputer algorithm contained a calibration procedure to calculate the temperature referable to a precision mercury-in-glass thermometer to an accuracy of ± 0.1°C. Cuvette temperature was maintained to within 0.1°C of the desired value using a Julabo refrigerated thermocirculator. In all experiments, temperature scans were performed from 2 to 45°C. The rate of warming (0.7 C° $\cdot$ min^{-1}) was controlled by the digital output of the microcomputer to the Julabo thermocirculator. Polarization was automatically monitored every 30 s and the results recorded on floppy disc for subsequent analysis. Polarization at specified temperatures were determined by interpolation of the resulting graphs. Possible artifacts due to the scattering of incident light or of the fluorescence emission [19] were assessed in parallel experiments (not shown), in which the effects of varying optical density on DPH polarization for both basolateral and brush-border membranes were found to be negligible.

Lipid extraction and fatty acid analysis

Lipids were extracted from membrane fractions using chloroform/methanol (2:1, v/v) containing 0.005% (w/v) butylated hydroxytoluene) as described previously [17]. The resulting lipid extracts were stored in sealed glass ampoules at −20°C under an atmosphere of nitrogen. Phospholipid classes were fractionated by two-dimensional thin-layer chromatography and phosphatidylcholine, phosphatidylethanolamine and serine/inositol phosphoglyceride classes were eluted and fatty acid methyl esters were prepared using boron trifluoride-methanol and analysed by gas-liquid chromatography all as described previously [17].

Results

Membrane fractionation procedure

Tables I and II record the recovery and specific activity of the various marker enzymes through the fractionation procedure. Fig. 2 illustrates the specific activities of each marker enzyme relative to the specific activity in the homogenate (purification factor) for the putative brush-border and basolateral fractions.

The total recovery of protein was approx. 95%. The recovery of the marker enzymes for the brush-border and basolateral membrane fractions, namely the alkaline phosphatase and Na^+/K^+-ATPase respectively, were 90%. This suggests that no appreciable losses of material occurred during the procedure and that the procedure had no great effect upon the functional properties of these enzymes.

Most of the acid phosphatase activity was lost in pellet P2b, whilst alkaline phosphatase and Na^+/K^+-ATPase showed a 3-fold purification in the upper fluffy part of the pellet (P2a). The brush-border membranes were purified from P2a by Mg^{2+}-precipitation and collected by centrifugation. The resulting pellet (P4) contained 26.5% of the original alkaline phosphatase activity in 4% of the original protein to give a 6.4-fold increase in specific activity over the original homogenate. Contamination by basolateral and lysosomal membranes was substantially reduced compared to the homogenate, since the relevant marker enzymes showed no increase in specific activity over the starting homogenate (Fig. 2) and very low recoveries. Succinate–cytochrome-*c* reductase activity was very low relative to the homogenate.

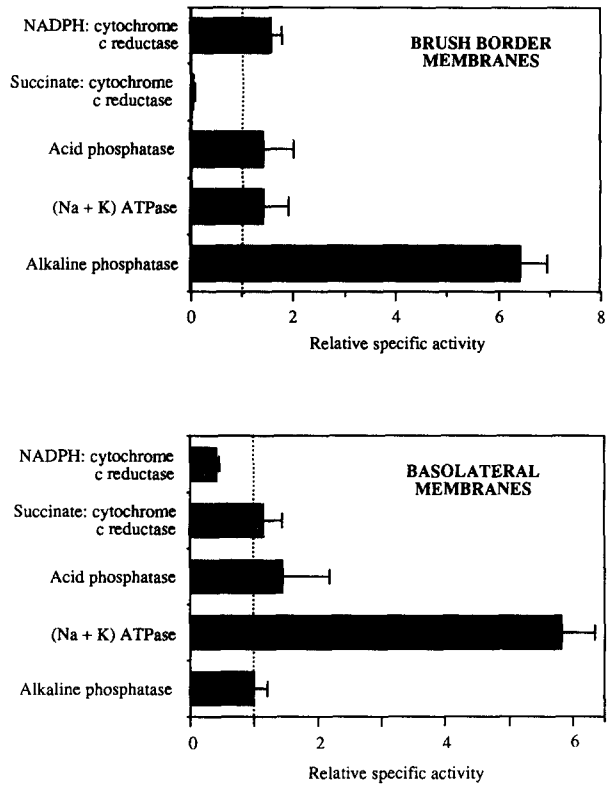

Fig. 2. The purification factor for membrane marker enzymes in putative brush-border (pellet P5) and basolateral membrane fractions (sucrose gradient fraction F5) from carp intestinal mucosa. Purification factors represent the increase in specific enzymatic activity over the starting homogenate. The dotted line indicates a purification factor of unity where the marker enzyme shows no change in specific activity compared to the starting homogenate.

TABLE I

Distribution of the microsomal-protein and marker-enzyme activities in membrane fractions obtained from carp intestinal mucosa by magnesium precipitation and sucrose density gradient centrifugation.

Values are % protein content or % total enzymatic activity of each fraction relative to the original homogenate reported as mean ± S.E. BBM represents the putative brush-border fraction and BLM the basolateral fraction. n.d., not detectable; tr, trace.

	n	P2a	P2b	S3	P3	P4 (BBM)	F1	F2 (BLM)	F3	F4	Total recovery
Protein	8	12.2±0.4	32.9±3.9	5.2±0.1	6.9±0.4	4.1±0.2	1.2±0.3	1.8±0.4	2.1±0.4	1.0±0.2	95.3±2.1
Alkaline phosphatase	5	37.3±3.0	30.9±2.2	29.5±2.0	5.4±1.9	26.4±2.4	0.9±0.3	2.2±0.7	2.2±1.1	tr	90.7±3.6
Na^+/K^+-ATPase	5	34.2±3.1	32.9±3.7	6.4±2.1	24.6±1.0	5.7±2.3	1.7±0.2	9.7±3.2	7.5±1.9	0.9±0.6	90.2±7.5
Acid phosphatase	4	14.0±6.6	75.6±5.5	6.1±2.5	8.2±4.7	5.5±2.1	1.4±0.7	1.6±0.1	1.7±1.2	0.6±0.4	108.0±4.1
Succinate–cytochrome-*c* reductase	3	8.0±1.8	18.3±5.1	0.2±0.1	5.4±1.3	tr	n.d.	1.0±0.3	1.6±0.3	1.3±0.4	74.7±3.8
NADPH–cytochrome-*c* reductase	3	19.9±3.3	18.6±6.9	7.2±0.9	8.8±0.9	6.2±0.4	1.2±0.6	1.8±0.9	1.2±0.4	0.2±0.2	74.5±5.3

TABLE II

Distribution of the marker enzymes in membrane fractions obtained from carp intestinal mucosa by magnesium precipitation and sucrose density gradient centrifugation

Values are $\mu mol \cdot min^{-1} \cdot g^{-1}$ microsomal protein and are reported as mean ± S.E. BBM represents the putative-brush-border fraction and BLM the basolateral fraction. n.d., not detectable.

	Homogenate	P2a	P2b	S3	S4	P4 (BBM)	F1	F2 (BLM)	F3	F4
Alkaline phosphatase	98.4 ± 4.2	299 ± 17.5	84.2 ± 8.5	558 ± 19.7	107 ± 30.3	629 ± 53.9	81.8 ± 19.7	96.3 ± 21.5	124 ± 42.6	19.5 ± 19.3
Na^+/K^+-ATPase	35.6 ± 3.9	99.1 ± 16.8	32.3 ± 1.5	43.6 ± 15.2	14.0 ± 2.3	51.0 ± 19.8	74.3 ± 26.4	209 ± 34.6	133 ± 2.1	52.9 ± 22.8
Acid phosphatase	46.7 ± 6.7	48.3 ± 21.3	135 ± 23.3	46.9 ± 17.9	37.9 ± 24.8	58.2 ± 22.9	97.6 ± 69.6	62.6 ± 28.7	56.9 ± 47.6	86.7 ± 52.6
Suecinate–cytochrome-*c* reductase	21.7 ± 1.3	13.5 ± 2.0	11.2 ± 3.0	0.8 ± 0.3	0.4 ± 0.2	0.8 ± 0.2	n.d.	18.5 ± 10.2	19.6 ± 4.3	50.2 ± 17.5
NADPH–cytochrome-*c* reductase	73.8 ± 3.8	121.0 ± 28.0	37.1 ± 11.7	99.8 ± 16.9	9.03 ± 0.7	115 ± 23.5	75.1 ± 14.5	17.6 ± 8.6	42.0 ± 8.3	21.3 ± 11.3

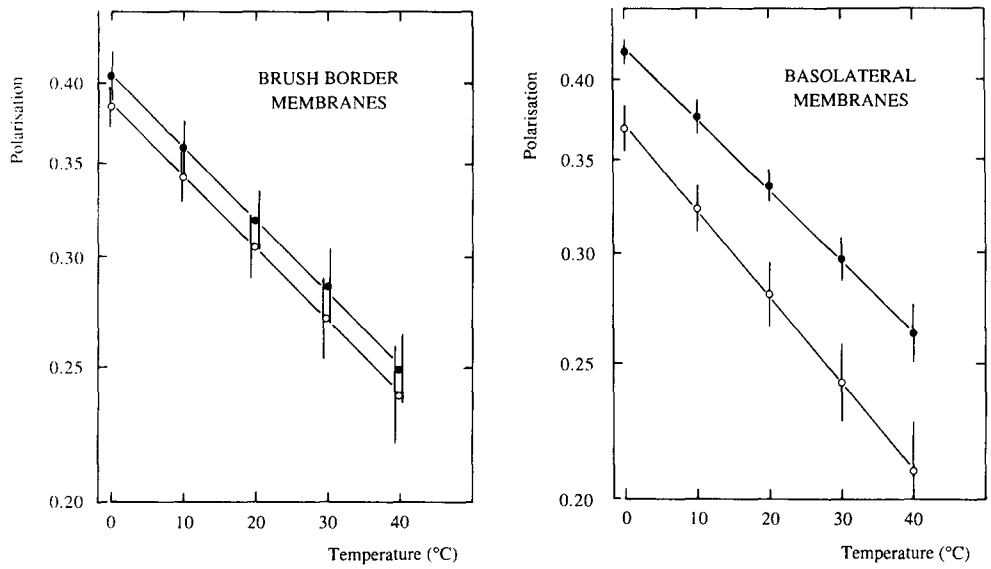

Fig. 3. Comparison of fluorescence polarization of DPH in brush-border and basolateral membranes of 10 °C(○)- and 30 °C (●)-acclimated carp. Values represent the means ± S.D., $n = 6$.

The pellet prepared from Mg^{2+}-precipitation was separated into 4 fractions on a discontinuous sucrose gradient. Fraction F2 (30/40% sucrose interface) was designated the basolateral fraction in view of its 5.8-fold increase in specific activity and high recovery (approx. 10%) of the Na^+/K^+-ATPase. Other marker enzymes showed low recoveries (1–2%) and no increases in specific activity (Fig. 2). Succinate–cytochrome-*c* reductase activity was undetectable.

DPH polarization

Fig. 3(a,b) shows temperature scans of DPH polarization in brush-border and basolateral membrane fractions from enterocytes of 10 and 30 °C-acclimated carp. All graphs were linear when plotted in semi-log form in contrast to the non-linear plots shown by other workers in corresponding membranes from mammals (for example, Ref. 20). These discontinuities have been frequently interpreted as evidence of thermotropic phase transi-

TABLE III

Distribution of the fatty acid composition (weight % ± S.E.) of the major phosphoglyceride classes of brush-border and basolateral fractions from intestinal mucosa of 10 and 30 °C-acclimated carp

	Phosphatidylcholine		Phosphatidylethanolamine	
	10 °C-acclimated ($n = 3$)	30 °C-acclimated ($n = 3$)	10 °C-acclimated ($n = 6$)	30 °C-acclimated ($n = 5$)
Brush border membranes				
$\Sigma_{saturated}$	38.8 ± 6.1	36.9 ± 4.8	42.7 ± 5.2	48.2 ± 5.4
$\Sigma_{mono\text{-}unsaturated}$	8.3 ± 3.0	17.9 ± 5.4	20.3 ± 4.2	10.9 ± 1.9
$\Sigma_{polyunsaturated}$	52.9 ± 11.8	45.2 ± 9.6	37.4 ± 4.3	40.9 ± 4.0
Saturation Ratio +	0.8 ± 0.2	0.6 ± 0.1	0.9 ± 0.2	1.1 ± 0.2
Unsaturation index ++	273 ± 21	247 ± 20	211 ± 26	216 ± 25
Basolateral membranes				
$\Sigma_{saturated}$	25.6 ± 1.4	38.3 ± 2.8	13.6 ± 3.8	39.1 ± 4.3
$\Sigma_{mono\text{-}unsaturated}$	15.0 ± 2.6	13.9 ± 2.1	9.7 ± 4.0	7.2 ± 1.5
$\Sigma_{polyunsaturated}$	59.5 ± 1.2	47.8 ± 1.8	76.7 ± 8.0	53.7 ± 2.2
Saturation ratio	0.3 ± 0.05	0.7 ± 0.1	0.2 ± 0.1	0.7 ± 0.1
Unsaturation index	324 ± 19	220 ± 19	392 ± 26	239 ± 25

+ Calculated as the $\Sigma_{saturated}$ divided by ($\Sigma_{mono\text{-}unsaturated} + \Sigma_{polyunsaturated}$).
++ Calculated as the Σ(wt % multiplied by the number of olefinic bonds).

tions, so their absence in the present studies may be evidence for their being no transition in these fish membranes over the temperature range 3–45 °C.

The brush-border fraction showed no statistically significant difference between the acclimation groups at any measurement temperature (Fig. 3a). The basolateral fraction, however, showed significantly lower DPH polarizations in 10 °C-acclimated fish compared to 30 °C-acclimated fish at all measurement temperatures ($P < 0.001$, Fig. 3b). This indicates that the basolateral membranes of cold-acclimated fish were distinctly more disordered, or more fluid, than the corresponding membranes of warm-acclimated fish. The magnitude of difference may be appreciated by calculating the 'homoeoviscous efficacy' [5] which was calculated by measuring the difference, in C °, of the position of the polarization/temperature curves for each acclimation group on the temperature axis and dividing by the difference in acclimation temperatures. The difference in position of the two curves for basolateral membranes was approx. 15 C °, which gives an efficacy of 0.75 or 75%. The equivalent value for the brush-border fraction was zero.

Acyl group composition

Table III summarizes the composition of the fatty acids found in the PC and PE classes of the brush-border-membrane fraction. In PC, the proportion of saturated fatty acids was rather similar in both acclimation groups. On the other hand, the proportion of mono-unsaturated fatty acids in 10 °C-acclimated fish was half that in 30 °C-acclimated fish and this corresponded in an increased proportion of polyunsaturated fatty acids in the latter. The PE there was a reduction of saturates in the cold which was mainly due to increased proportions of mono-unsaturates. Two indices of fatty acid composition have been calculated, the ratio of saturated to unsaturated fatty acids (the saturation ratio) and an unsaturation index (calculated as the sum of the weight % multiplied by the number of unsaturated bonds for each component of the mixture of fatty acids, see Ref. 21). The differences between acclimation groups had no great effect upon the indices of overall composition. Thus, the unsaturation index was almost identical in 10 °C- and 30 °C-fish for both phospholipid classes. The saturation ratio, which was calculated as the ratio of saturated to unsaturated fatty acids, also showed no significant differences between acclimation groups.

Table III also shows equivalent data for the basolateral fraction. In this case, temperature acclimation produced much larger changes in fatty acid composition. In both phospholipid classes the proportion of saturated fatty acids was substantially reduced in 10 °C-acclimated fish relative to 30 °C-acclimated fish and this corresponded mainly to an increase in the proportion of polyunsaturated fatty acids. The mono-unsaturated fatty acids were largely unaffected. The result was a 50% increase in unsaturation index and a substantial reduction in saturation ratio.

Discussion

Ca^{2+}-precipitation is a widely used technique for the purification of brush-border membranes from mammalian tissue [22,23], although the substitution of Mg^{2+} for Ca^{2+} has recently been introduced in order to reduce phospholipase activity [11]. The purification scheme described here uses the Mg^{2+}-precipitation technique at a previously untried stage, together with an increased centrifugation speed of $9000 \times g$ to pellet a greater proportion of contaminating membranes [24]. The brush-border fraction was recovered by high speed centrifugation of the resulting supernatant. The basolateral membrane fraction was recovered from the pellet arising from Mg^{2+}-precipitation using the discontinuous sucrose gradient described by other authors [13,25]. The 'Percoll' technique [26], in our hands, failed to produce the readily separated bands described by these authors.

The fractionation scheme described here provided brush-border and basolateral membranes of reasonable purity from the same homogenate. The technique has the advantage of being relatively simple, rapid and reproducible. Both fractions showed limited cross-contamination by each other and the marker enzymes for mitochondria, lysosomes and endoplasmic reticulum showed no enrichment of activity relative to the starting homogenate, indicating again only limited contamination. The purification factors for the marker enzymes of both the brush-border and basolateral membranes fractions were somewhat lower than those observed in mammalian tissues (10–30-fold [27–29]) but were similar to those found in studies of lower vertebrates (4–13-fold [11,23,30]). Comparisons of fluidity of fractionated membranes from mammalian transporting epithelia have concluded that brush borders are less fluid than basal membranes [31–33]. However, the present results show little difference in the polarization of DPH in brush-border and basolateral membranes in carp intestine.

DPH polarization of basolateral membrane preparations from 10 °C-acclimated fish was substantially lower than corresponding values for 30 °C-acclimated fish, the difference being highly significant at all measurement temperatures. The size of the difference between acclimation groups can be conveniently gauged by measuring the extent to which it offsets the direct ordering effects of the temperature difference, to give the so-called 'homoeoviscous efficacy' (HE). For the basolateral membranes HE was 75%, a value that is the highest recorded in eukaryote membranes with the polarization technique [34]. By contrast, DPH polarization in the brush-border fraction was not affected by

temperature-acclimation because HE was not significantly different from zero. Thus, despite the fact that the two fractions were obtained from the same populations of cells, they display quite different responses to temperature acclimation.

Different homoeoviscous responses of membrane fractions isolated from the same tissue have been recorded previously in liver microsomal and mitochondrial fractions of the green sunfish [35] and in brain myelin and synaptic fractions of goldfish [36]. However, the contrast in responses of the two intestinal fractions were considerably greater than in these other tissues. The lack of homoeoviscous adaptation, as observed here in the brush-border fraction, is unusual and has been documented previously only in the sarcoplasmic reticulum of goldfish muscle [37]. The brush-border membranes showed some changes in acyl group composition of the major phospholipid classes, though as with the sarcoplasmic reticulum, these were largely limited to changes in the proportion of mono- and polyunsaturated fatty acids. The ratio of saturated to unsaturated fatty acids, or more simply the proportion of saturated fatty acids, is thought to be a good indicator of the physical properties of the membrane and of homoeoviscous adaptation [21]. In brush-border membranes these were found to be unaffected by temperature acclimation, at least in comparison to the changes in the basolateral fraction. By contrast, in the basolateral membrane fraction a significant decrease in saturation on cold acclimation was observed in both phosphoglyceride classes and this was reflected by comparatively large changes in both indices of lipid composition. Whilst these indices do not take account of all important structural features of fatty acid structure which relate to measured membrane order [21], they clearly correlate in direction with the changes in DPH polarization in basolateral membranes and to that extent confirm the polarization measurements.

Thus, comparison of basolateral membrane fractions from cold- and warm-acclimated fish has shown significant and correlated differences in DPH polarization and in fatty acid composition, whilst the brush-border fraction appeared to be virtually unaffected by acclimation. These conclusions are consistent with the proposed adaptations of intestinal transport in temperature-acclimated carp [3]. Adaptation of the Na^+-pump, brought about through a viscotropic effect by changes in the molecular order of the surrounding bilayer, might be a particularly effective means of maintaining the transmembrane concentration gradient for Na^+ and thereby influencing the properties of a number of Na^+-dependent transport systems [1].

Examples of adaptive change in the brush border of fish and rat intestinal mucosa to dietary manipulation have been reported [11,38,39]. The apparent lack of adaptive change in the brush-border fraction during temperature acclimation, therefore, may be specific to temperature acclimation. This may be rationalized either by assuming that the transport properties specific to the brush border are not sensitive to the molecular order of the bilayer as defined by DPH polarization, or that transport across the brush-border membrane is not rate-limiting to transepithelial transport as a whole. Neither of these assumptions is unreasonable, especially as others [11] have noted that although the permeability properties of trout brush-border membranes were affected by dietary manipulation, there was no correlated change in DPH polarization. A change in molecular order of the brush-border membrane would not necessarily influence the transport properties of the epithelium.

The differential responsiveness of apical and basal membranes of the intestinal mucosa provides evidence for a highly sophisticated cellular regulation of the biochemical composition and physicochemical structure of the different cellular membrane systems. This not only elicits the necessary adaptive responses in cellular performance but may also avoid the potentially high energetic costs associated with a restructuring of the brush-border membrane when no adaptive advantage would result.

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