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PRESERVATION OF GLUCOSE TRANSPORT AND ENZYME ACTIVITY IN FISH INTESTINAL BRUSH-BORDER AND BASOLATERAL MEMBRANE VESICLES

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Summary

The preservation of glucose transport and membrane-bound enzyme activity in intestinal brush-border and basolateral membrane vesicles from the euryhaline teleost, *Oreochromis mossambicus*, was examined. Transport was well-preserved when the vesicles were either frozen in liquid nitrogen or lyophilized and stored in the presence of trehalose. Transport activity was not well-maintained when vesicles were lyophilized with mannitol as the protective carbohydrate. Enzyme activities were reduced by liquid nitrogen storage or by lyophilization with mannitol. However, enzyme enrichments of all treatments remained fairly constant over storage time, suggesting similar functional and perhaps structural changes in the different membrane fractions in response to preservation. These techniques provide a simple way to obtain functionally similar vesicles over a relatively long period of storage, permitting improved comparisons of transport properties. These results extend for the first time to vertebrate epithelial membranes the finding that trehalose preserves transport activity during lyophilization, storage and rehydration.

Introduction

Techniques for the separation of various cellular membrane fractions have led to significant advances in understanding their different transport and enzymatic properties. However, transport processes and enzyme activities of isolated membranes are not generally found to be well-preserved with time even when stored on ice. For this reason membrane vesicles are usually prepared on the experimental day and used fresh. It is desirable to be able to preserve these samples for a number of reasons. The combined time for membrane preparation and assay is often quite long and this limits the number of treatments possible on a particular sample owing to progressive deterioration. In addition, the ability to preserve the transport characteristics of a large quantity of vesicles permits many

Key words: glucose transport, membrane vesicles, brush border, fish intestine.

experiments on the same vesicle population, thereby eliminating a significant source of experimental variation and facilitating comparison of results.

Several laboratories have reported the preservation of various transport and enzyme activities in isolated membranes by low-temperature storage (Ahmed *et al.* 1976; Hittelman *et al.* 1978; Stevens *et al.* 1982) and by lyophilization (Crowe & Crowe, 1982; Mouradian *et al.* 1985). In low-temperature studies on intestinal and kidney membrane preparations (Hittleman *et al.* 1978; Stevens *et al.* 1982), fresh glucose transport characteristics were not well-preserved and exhibited variable modifications induced by the preservation process (e.g. increase or decrease in initial uptake rate and overshoot). The ability of membrane preparations to maintain structural and functional integrity during lyophilization has only been measured using lobster muscle sarcoplasmic reticulum (SR), the measurement of physiological activity in these SR studies being defined by transmembrane calcium fluxes (Crowe & Crowe, 1982; Mouradian *et al.* 1985). However, such preservation could only be achieved by the inclusion of specific carbohydrates which prevented damage to the membrane (Crowe *et al.* 1984*b*), trehalose, a disaccharide of glucose, being the most protective.

In this paper we report experiments on the preservation of glucose transport and enzyme activities during freezing and lyophilization of brush-border membrane vesicles (BBMV) and basolateral membrane vesicles (BLMV) from the upper and lower intestinal epithelium of the warm-water euryhaline fish, *Oreochromis mossambicus*.

Materials and methods

Preparation of vesicles

African tilapia (*Oreochromis mossambicus*; each approx. 100 g wet mass) were collected from local Hawaiian waters and kept overnight in clean, aerated sea water. Fish were killed by a blow to the head. Intestines were removed and divided into upper and lower segments; the upper being defined as the anterior half, and the posterior one-third making up the lower intestinal segment. Brush-border membrane vesicles (BBMV) and basolateral membrane vesicles (BLMV) were prepared from the intestinal segments as previously described (Reshkin & Ahearn, 1987*a*,*b*). Purified membranes were resuspended in sufficient buffer (300 mmol l^{-1} mannitol, 12 mmol l^{-1} Hepes-Tris, pH 7·4) to provide a final vesicle protein concentration of 7–10 mg ml^{-1}. Protein concentrations were assessed with the Bio Rad protein assay using bovine serum albumin as standard.

Preservation and storage techniques

Samples of tissue homogenate and purified vesicles (0·5–1 ml) were transferred to cryotubes, quickly frozen in liquid nitrogen and either kept in the liquid nitrogen or immediately lyophilized and stored under vacuum in the dark. When trehalose was to be included in the preparation it was added just after the final vesicle resuspension by addition of a small volume of a concentrated solution

(1 mol l^{-1}) to a final concentration in the preparation of 1 mg trehalose per mg of membrane. The sample was then treated as described above. At appropriate times, samples were either thawed slowly on ice or rehydrated to their original volume with distilled water, and then resuspended by 10 passes through a 22 gauge needle.

Enzyme assays

The relative purity of membrane preparations is often assessed by comparing the enrichments of various membrane marker enzymes throughout the preparation process with those of the initial tissue homogenate. Alkaline phosphatase activity was determined using Sigma kit no. 104, leucine aminopeptidase activity was measured by the technique of Haase *et al.* (1978), and cytochrome *c* oxidase activity was assayed following the method of Cooperstein & Lazarow (1951). Na^+/K^+-stimulated ATPase was measured by the coupled reaction assay of Berner *et al.* (1976).

Transport measurements

Transport studies using intestinal BBMV and BLMV were conducted at 20°C using the Millipore filtration technique of Hopfer *et al.* (1973). For D- and L-glucose transport in BLMV a rapid uptake apparatus (Innovativ Labor AG; Adliswil, Switzerland) was used which allowed automatic control of the incubation period to 1 s. Transport of D-[^{3}H]- or L-[^{3}H]glucose into BLMV was initiated by mixing 5 μl of membrane suspension with 20 μl of radiolabelled incubation medium containing 300 mmol l^{-1} mannitol, 12 mmol l^{-1} Hepes-Tris and 1 mmol l^{-1} D- or L-glucose, pH 7·4. Transport was terminated by injecting 2 ml of ice-cold stop solution into the uptake mixture (stop solution composition was the same as incubation medium except that 1 mmol l^{-1} phloretin replaced the glucose.)

For BBMV, D-[^{3}H]glucose uptake was initiated by mixing 20 μl of membrane suspension with 180 μl of radiolabelled incubation medium containing 0·1 mmol l^{-1} unlabelled D-glucose. Incubation medium contained 150 mmol l^{-1} NaCl, KCl or NaCl plus 0·1 mmol l^{-1} phloridzin and 12 mmol l^{-1} Hepes-Tris, pH 7·4. D-Glucose uptake was terminated by injecting 20 μl of the uptake mixture into 2 ml of ice-cold stop solution (of the same composition as the incubation medium, except lacking glucose).

After transport had been terminated, the vesicle samples were immediately filtered onto a Millipore filter (0·65 μm) and washed with another 10 ml of ice-cold stop solution. The filters, containing the vesicles and their associated radiolabelled glucose, were placed in Beckman Ready Solv scintillation cocktail and counted in a Beckman LS-8100 scintillation spectrometer.

To determine nonspecific binding, all BBMV transport values were corrected for a 'vesicle blank', obtained by adding the incubation medium and vesicles directly to the stop solution prior to filtering, extracting in scintillation cocktail, and counting. For experiments with BLMV, initial transport rates (influxes) were calculated from the slope of the linear portion (up to 8 s) of the uptake curve. In

BLMV, nonspecific glucose binding was estimated by extrapolating the linear portion of the uptake curve to zero time.

All results were expressed as means ± standard error of 3–5 replicates. Paired or unpaired *t*-tests were used for all statistical analyses. Standard error bars on figures have been omitted because they were smaller than the symbols.

Reagent grade chemicals and the inhibitors phloridzin and phloretin were purchased from Sigma Chemical Co. Radioactive D- and L-glucose were purchased from New England Nuclear Corporation.

Results

Effects of storage on enzyme activities

Total enzyme activities of both homogenates and vesicles stored in liquid nitrogen decreased with time (Tables 1 and 2). However, the relative change between homogenate and vesicles was fairly constant as reflected by the similar purification factors found at all times for both BBMV and BLMV preparations. The preservation of enzyme activity in lyophilized BLMV samples was very similar, over short periods, to that seen for identical vesicles stored in liquid nitrogen. Samples stored for longer periods after lyophilization (216 h) showed greater loss of activity than samples stored in liquid nitrogen. As for samples stored in liquid nitrogen, the enrichment factors for lyophilized vesicles were not significantly different from those of fresh samples, again suggesting that degradation of homogenate and vesicles was similar.

Table 1. Enzymatic characterization of tilapia (Oreochromis mossambicus) *upper and lower intestinal brush-border membrane vesicles*

Enzyme	Preservation time (h)*	Activity: Homogenate Upper	Activity: Homogenate Lower	Activity: Vesicles Upper	Activity: Vesicles Lower	Purification factor Upper	Purification factor Lower
Alkaline phosphatase	0	14 ± 1·3	6 ± 0·3	258 ± 8·6	79 ± 1·9	18·4 ± 0·6	13·2 ± 0·7
	48	12 ± 1·4	5 ± 0·5	206 ± 6·1	63 ± 2·4	17·2 ± 0·5	12·6 ± 0·3
	216	8 ± 1·0	4 ± 0·3	150 ± 3·1	48 ± 1·6	18·7 ± 0·6	12·7 ± 0·5
Leucine amino-peptidase	0	25 ± 1·8	20 ± 1·8	209 ± 5·4	181 ± 3·2	8·4 ± 0·5	9·0 ± 0·3
	48	18 ± 2·1	16 ± 1·3	167 ± 4·2	136 ± 2·7	9·3 ± 0·8	8·5 ± 0·4
	216	17 ± 0·9	17 ± 1·2	93 ± 1·3	90 ± 1·8	5·5 ± 0·6	5·3 ± 0·4
Na^+/K^+-ATPase	0	45 ± 3·4	35 ± 2·7	42 ± 1·1	37 ± 1·2	0·9 ± 0·1	1·1 ± 0·3
	48	31 ± 3·0	26 ± 1·9	36 ± 0·8	22 ± 0·6	1·2 ± 0·2	0·8 ± 0·1
	216	30 ± 2·8	26 ± 2·5	32 ± 1·4	24 ± 0·9	1·1 ± 0·1	0·9 ± 0·1

* Time in liquid nitrogen.
Values are means ± S.E.
Enzyme activities are μmol product released mg protein^{-1} h^{-1}.
Purification factors are means of individual vesicle activities ÷ individual homogenate activities.

Table 2. *Enzymatic characterization of tilapia* (Oreochromis mossambicus) *upper and lower intestinal basolateral membrane vesicles*

Enzyme	Preservation time (h)*	Activity: Homogenate Upper	Homogenate Lower	Vesicles Upper	Vesicles Lower	Purification factor Upper	Purification factor Lower
Na^+/K^+-ATPase	0	5·2 ± 0·3	7·3 ± 0·5	62·0 ± 3·4	81·3 ± 2·9	11·4 ± 0·9	11·7 ± 0·4
	48 (L)	3·1 ± 0·5	4·7 ± 0·4	39·1 ± 2·6	57·5 ± 3·7	12·7 ± 0·4	11·8 ± 0·6
	72 (f)	4·3 ± 0·6	5·5 ± 0·3	47·3 ± 2·2	66·1 ± 3·9	11·0 ± 0·5	12·4 ± 0·7
	216 (L)	2·2 ± 0·3	3·4 ± 0·2	29·4 ± 1·9	40·4 ± 2·8	13·4 ± 0·6	12·1 ± 0·5
	240 (f)	3·6 ± 0·6	4·9 ± 0·5	41·2 ± 2·5	62·3 ± 3·1	12·3 ± 0·6	12·5 ± 0·4
Alkaline phosphatase	0	24·6 ± 1·3	13·2 ± 0·9	12·3 ± 1·6	9·2 ± 1·3	0·4 ± 0·02	0·7 ± 0·06
	48 (L)	17·2 ± 1·6	9·4 ± 0·7	6·9 ± 0·8	4·2 ± 0·2	0·6 ± 0·04	0·4 ± 0·02
	72 (f)	18·7 ± 1·4	10·2 ± 0·8	6·7 ± 1·1	5·3 ± 0·4	0·4 ± 0·07	0·5 ± 0·03
	216 (L)	11·2 ± 1·1	5·4 ± 0·4	7·9 ± 0·9	3·6 ± 0·4	0·7 ± 0·05	0·6 ± 0·04
	240 (f)	15·4 ± 1·3	8·9 ± 0·8	7·7 ± 1·2	6·4 ± 0·8	0·5 ± 0·07	0·7 ± 0·08
Leucine amino-peptidase	0	14·3 ± 1·1	16·8 ± 1·4	3·6 ± 0·4	5·4 ± 0·6	0·2 ± 0·07	0·3 ± 0·05
	48 (L)	11·1 ± 1·3	11·9 ± 0·9	6·7 ± 0·9	4·8 ± 0·3	0·6 ± 0·04	0·4 ± 0·03
	72 (f)	10·8 ± 1·3	11·3 ± 1·5	4·3 ± 0·5	4·9 ± 0·7	0·4 ± 0·05	0·4 ± 0·06
	216 (L)	7·6 ± 0·6	6·9 ± 0·7	1·9 ± 0·3	2·4 ± 0·2	0·2 ± 0·04	0·2 ± 0·02
	240 (f)	9·5 ± 0·5	10·4 ± 1·1	4·4 ± 0·5	6·2 ± 0·08	0·5 ± 0·05	0·6 ± 0·07

Values are means ± S.E.

Enzyme activities are in μmol product released mg protein^{-1} h^{-1}.

f, freezing in liquid nitrogen; L, lyophilization.

Purification factors are means of individual vesicle activities (30/40 sucrose gradient fraction) ÷ individual homogenate activities.

Preservation of glucose transport

The time courses of glucose uptake for upper and lower intestinal BBMV for fresh vesicles and for those after 48 and 216 h of storage in liquid nitrogen are presented in Figs 1 and 2, respectively. Freezing of BBMV in liquid nitrogen conserved the properties of glucose transport found in fresh vesicles, including sodium-dependency of upper and lower segments, overshoot characteristics of the upper intestine, and phloridzin inhibition of carrier-mediated transport. In addition, the quantitative equilibrium space values at 60 min of uptake (upper: 116 ± 3·7; lower: 280 ± 5·9 pmol mg protein^{-1}; means of fresh and preserved vesicles ± S.E.) were also uninfluenced by liquid nitrogen preservation, as were apparent initial transport rates calculated from 15 s uptake values (upper: 6·7 ± 0·2; lower: 4·5 ± 0·3 pmol mg protein^{-1} s^{-1}).

Table 3 shows the effect of lyophilization of BBMV in the presence of 1:1 trehalose:membrane (w/w) on the preservation of D-glucose transport activity for a maximum period of 312 h. During this period there was no loss of functional activity of these BBMV. Sodium-dependency of D-glucose uptake for both upper and lower intestinal segments, apparent initial transport rates calculated from 8 s

Table 3. *Stabilizing effects of trehalose on the transport activity of brush-border membrane vesicles (upper and lower intestine) subjected to lyophilization*

	0 h		48 h		216 h		312 h	
	Na^+	K^+	Na^+	K^+	Na^+	K^+	Na^+	K^+
Upper intestine								
Apparent initial rate of transport	5·1 ± 0·3	1·2 ± 0·1	5·9 ± 0·4	1·4 ± 0·1	5·7 ± 0·6	1·3 ± 0·1	5·3 ± 0·4	1·3 ± 0·1
Overshoot	1·52	—	1·71	—	1·58	—	1·62	—
Equilibrium	50 ± 15	47·2 ± 4	46·7 ± 9	54 ± 3	49·3 ± 5	51·5 ± 6	47·7 ± 3	51·2 ± 3
Lower intestine								
Apparent initial rate of transport	4·6 ± 0·4	1·3 ± 0·1	5·2 ± 0·3	1·5 ± 0·1	4·9 ± 0·5	1·4 ± 0·1	5·0 ± 0·4	1·4 ± 0·1
Equilibrium	122 ± 5	118 ± 9	127 ± 3	116 ± 2	126 ± 5	120 ± 10	124 ± 10	119 ± 5

Values are means ± S.E.
Uptake rates are pmol mg protein^{-1} s^{-1} and equilibrium values are pmol mg protein^{-1}.
Overshoot is the ratio of the 1 min uptake value to the equilibrium uptake value.
Ratio of trehalose to membrane was 1:1 by weight.

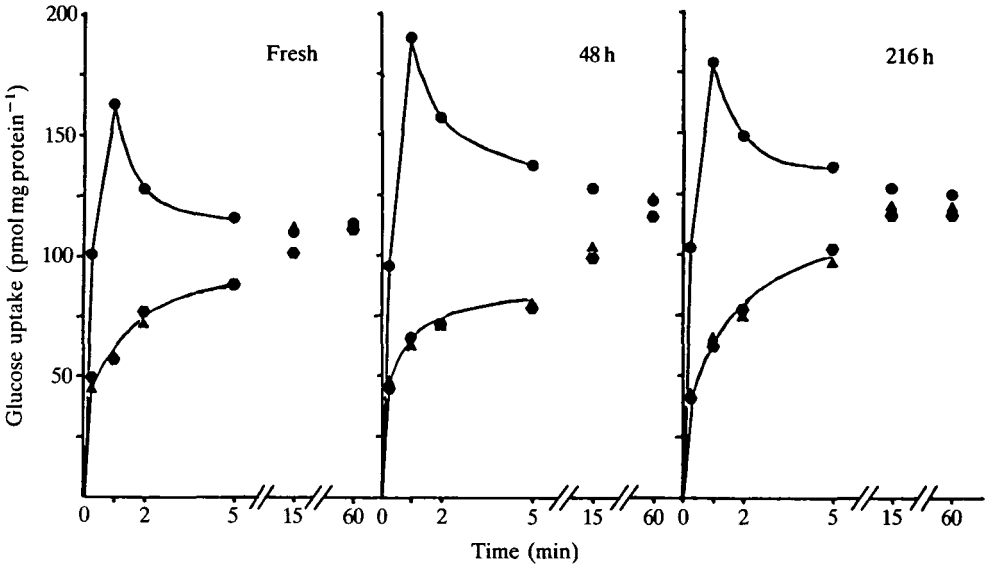

Fig. 1. Time course of 0·1 mmol l^{-1} D-[^{3}H]glucose uptake by upper intestinal brush-border membrane vesicles of seawater tilapia freshly prepared and frozen in liquid nitrogen for 48 and 216 h. Incubation medium contained 120 mmol l^{-1} NaCl (circles), 120 mmol l^{-1} KCl (triangles) or 120 mmol l^{-1} NaCl plus 0·1 mmol l^{-1} phloridzin (hexagons). Symbols represent means of 3–4 replicates per time interval. Values of S.E.M. were smaller than the symbols.

uptake values (upper: 5·1; lower: 4·6 pmol mg protein^{-1} s^{-1}), overshoot characteristics of the Na^+-stimulated D-glucose uptake in the upper intestine, and the quantitative equilibrium space values at 60 min of uptake were almost identical to those recorded for fresh membrane preparations.

Carrier-mediated D-glucose uptake in BLMV was estimated as the difference between initial rates (0–8 s) of D- and L-glucose uptake. Fig. 3 shows the representative time course for BLMV glucose uptake from fresh preparations. Both D- and L-glucose uptakes were linear up to 8 s and calculated nonspecific binding values, estimated from extrapolation to zero time (upper: 26·7 ± 1·0; lower: 110 ± 0·8 pmol mg^{-1}), were not significantly different ($P > 0·05$) for the two molecules. Table 4 presents the uptake parameters of fresh, frozen and lyophilized (without trehalose) BLMV preparations. Storage of BLMV frozen in liquid nitrogen elicited no change in carrier-mediated uptake, nonspecific binding or equilibrium value for storage of up to 240 h. Lyophilized storage in the presence of mannitol, however, did not preserve well the functional activity of the membrane compared with that of fresh preparations. Even after only 48 h of storage, rates of total and carrier-mediated uptake and respective equilibrium values were reduced by approximately 50 % from those of fresh preparations. There was also a small, but significant ($P < 0·05$), decrease in nonspecific binding. After 216 h of lyophi-

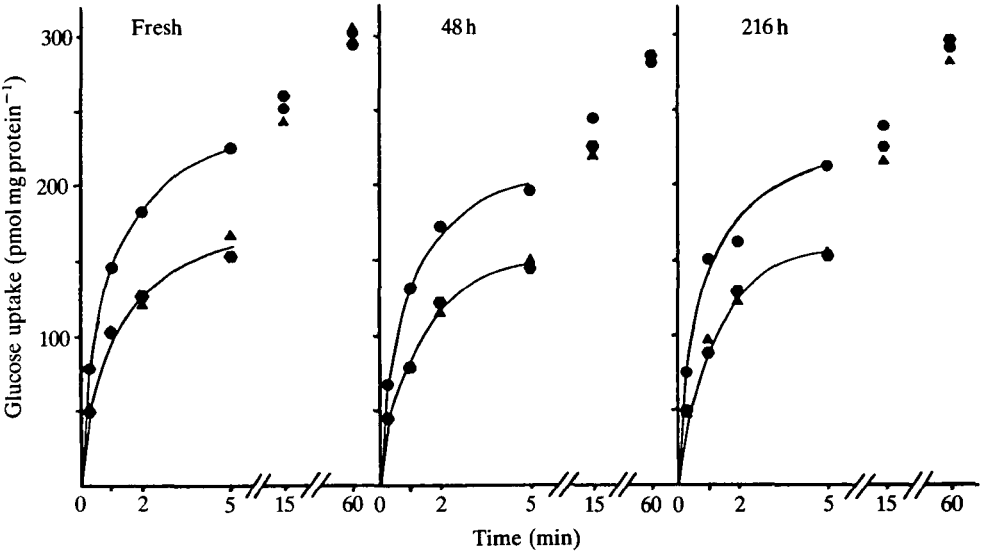

Fig. 2. Time course of 0·1 mmol l^{-1} D-[^{3}H]glucose uptake by lower intestinal brush-border membrane vesicles of seawater tilapia freshly prepared and frozen in liquid nitrogen for 48 and 216 h. Incubation medium contained 120 mmol l^{-1} NaCl (circles), 120 mmol l^{-1} KCl (triangles) or 120 mmol l^{-1} NaCl plus 0·1 mmol l^{-1} phloridzin (hexagons). Symbols represent means of 3–4 replicates per time interval.

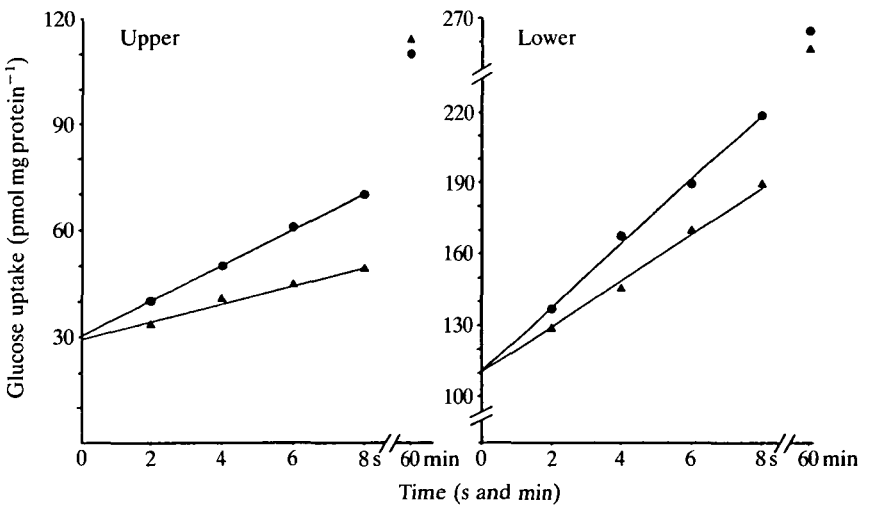

Fig. 3. Time course of 1 mmol l^{-1} D-[^{3}H]glucose (circles) and L-[^{3}H]glucose (triangles) uptake by freshly prepared basolateral membrane vesicles from the upper and lower intestine of seawater tilapia. Symbols represent means of 3–4 replicates per time interval.

Table 4. *Effects of freezing and lyophilization (no trehalose) on transport characteristics of tilapia* (Oreochromis mossambicus) *upper and lower intestinal basolateral membrane vesicles*

	Rate of glucose uptake			
	D-glucose	L-glucose	D-glucose − L-glucose	Equilibrium value
Fresh				
Upper	5·5 ± 0·3	3·5 ± 0·4	2·1 ± 0·5	110 ± 6·7
Lower	13·0 ± 0·9	10·7 ± 0·6	2·2 ± 0·7	267 ± 8·9
Lyophilized (48 h)				
Upper	2·6 ± 0·4	1·4 ± 0·2	1·1 ± 0·3	52 ± 5·2
Lower	4·7 ± 0·3	3·4 ± 0·6	1·4 ± 0·5	138 ± 7·6
Frozen (72 h)				
Upper	5·1 ± 0·3	2·8 ± 0·2	2·3 ± 0·7	102 ± 4·1
Lower	9·0 ± 0·5	6·5 ± 0·7	2·4 ± 0·6	270 ± 7·5
Lyophilized (216 h)				
Upper	2·2 ± 0·7	1·5 ± 0·8	0·9 ± 0·7	40 ± 3·3
Lower	0·9 ± 1·2*	0·8 ± 0·9*	0·2 ± 0·2*	105 ± 6·4
Frozen (240 h)				
Upper	5·2 ± 0·6	3·1 ± 0·3	2·2 ± 0·5	105 ± 2·8
Lower	8·2 ± 0·7	5·8 ± 0·7	2·4 ± 0·7	245 ± 7·2

Values are means ± S.E.
Uptake rates are pmol mg protein^{-1} s^{-1} and equilibrium values are pmol mg protein^{-1}.
* Values not significantly different from zero.

lized storage, the uptake characteristics had decayed even further in upper intestinal BLMV and were not significantly different from zero in lower intestinal BLMV.

To determine whether the inclusion of trehalose with the membranes would preserve BLMV glucose transport during lyophilization, trehalose was added to the vesicles in a 1:1 ratio to membrane (w/w). Table 5 presents some of the uptake parameters from fresh and lyophilized BLMV preparations. Addition of trehalose prior to lyophilization resulted in preservation of BLMV uptake properties, including total uptake, carrier-mediated uptake, nonspecific binding and equilibrium value for periods up to 312 h.

Fig. 4 presents a summary of the effects of lyophilization on glucose transport in tilapia intestinal BBMV (with trehalose) and BLMV (with and without trehalose). Data are presented as a percentage of the initial glucose transport rate in fresh vesicle preparations for each sampling period after lyophilization. The initial transport rate of the BLMV lyophilized without trehalose dropped rapidly compared with the initial rates in the fresh vesicles, whereas the initial rates in both BBMV and BLMV lyophilized in the presence of trehalose were comparable

Table 5. *Effects of lyophilization in the presence of trehalose on transport characteristics of tilapia* (Oreochromis mossambicus) *upper and lower intestinal basolateral membrane vesicles*

	Rate of glucose uptake			
	D-glucose	L-glucose	D-glucose− L-glucose	Equilibrium value
Fresh				
Upper	4·8 ± 0·3	2·3 ± 0·6	2·5 ± 0·2	125 ± 8·2
Lower	14·6 ± 1·1	12·4 ± 0·9	2·2 ± 0·3	237 ± 6·9
48 h				
Upper	4·6 ± 0·2	2·4 ± 0·3	2·2 ± 0·5	112 ± 7·7
Lower	14·8 ± 0·9	12·7 ± 1·0	2·1 ± 0·3	243 ± 9·1
216 h				
Upper	4·9 ± 0·5	2·2 ± 0·1	2·7 ± 0·5	117 ± 6·5
Lower	14·5 ± 1·4	12·2 ± 1·1	2·3 ± 0·2	221 ± 7·3
312 h				
Upper	4·9 ± 0·6	2·3 ± 0·4	2·6 ± 0·1	109 ± 7·3
Lower	14·8 ± 0·9	12·6 ± 1·3	2·2 ± 0·4	231 ± 8·6

Values are means ± S.E.
Uptake rates are pmol mg protein^{-1} s^{-1} and equilibrium values are pmol mg protein^{-1}.
Ratio of trehalose weight to total membrane weight was 1:1.

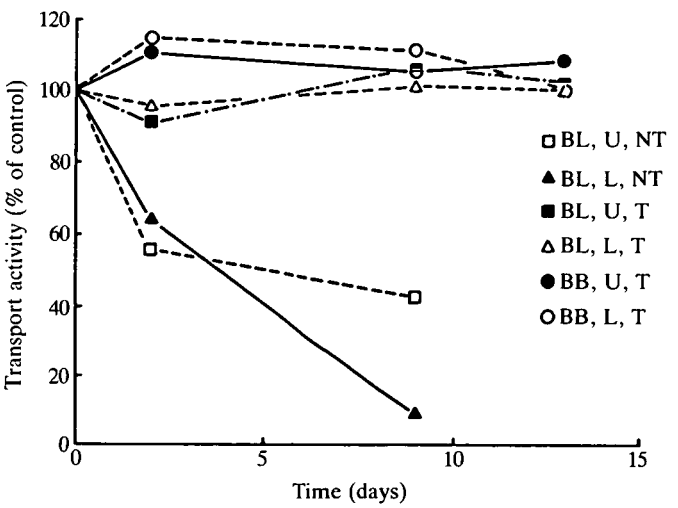

Fig. 4. Glucose uptake in rehydrated tilapia intestinal BBMV (BB) and BLMV (BL) from upper (U) and lower (L) intestine previously dried with trehalose (T) or without trehalose (NT).

to those found in the fresh preparations. These data support, and extend to vertebrate intestinal BBMV and BLMV, the protective effect of trehalose on biological membranes when they are subjected to lyophilization and reconstitution.

Discussion

The present research has examined the effects of two preservation techniques, freezing and lyophilization, on intestinal BBMV and BLMV enzyme activities and glucose transport characteristics over extended periods. Long-term storage and maintenance of functional integrity of a membrane system offers some advantages over exclusive use of fresh preparations. The yield upon reactivation of preparations similar, or identical, in activity to fresh vesicles greatly reduces experimental time, increasing productivity. Additionally, preservation of a single large preparation allows an increased number of experiments to be conducted on the same vesicle population, eliminating variability due to organismal and preparative differences. Furthermore, when using organisms or tissues difficult to obtain or maintain, the ability to preserve preparation viability greatly facilitates experimentation.

We found a general decline in total homogenate and vesicle enzyme activities in both frozen and lyophilized samples. The decline of enzyme activities in these different membrane fractions was, however, about the same as reflected in the general consistency of the enrichment factors over time. Liquid nitrogen storage was a better preservation treatment for BLMV than was lyophilization (see Table 2). These data suggest that protein activity was maintained similarly in homogenate and vesicle preparations and that various structural changes contributing to functional loss of enzyme activity were probably the same in the two preparative steps.

Our results clearly show that rapid freezing and storage in liquid nitrogen (up to 216 h for BBMV and 240 h for BLMV), in conjunction with slow, gentle thawing of the samples on ice, results in a vesicle preparation with essentially identical glucose transport characteristics to those found in the fresh preparation. In BBMV, initial uptake rate, overshoot (in the upper intestine) and equilibrium value were all conserved essentially unchanged. This is in contrast to the results of previous studies. Hittelman *et al.* (1978) attempted to preserve D-glucose transport in mammalian kidney BBMV by freezing at −70 °C in glycerol solutions. Glucose overshoots were lost and equilibrium values were increased by 25–50 %. Although more successful at preserving glucose uptake characteristics in mammalian intestinal BBMV and BLMV, and in kidney BBMV, the results of Stevens *et al.* (1982) are extremely variable. In their study quick freezing and storage in liquid nitrogen resulted in an almost 100 % increase in initial uptake rate and overshoot in intestinal BBMV, a 100 % increase in glucose uptake rate and equilibrium value in intestinal BLMV, and a general decline over time in all glucose uptake

properties in renal BBMV. Thus, similar techniques of preservation did not result in as complete a maintenance of vesicle glucose transport properties as shown in this study.

The major difference in preservation technique between the present research and the two studies cited above is the way in which the frozen vesicles were thawed. In previous studies (Hittelman *et al.* 1978; Stevens *et al.* 1982), the vesicles were thawed rapidly at relatively high temperature (37 °C and 40 °C, respectively), whereas we thawed the frozen vesicles slowly on ice. This slow thawing presumably results in less structural and, therefore, functional change in membranes and associated proteins, resulting in an improved preservation of original transport properties (Rudolph & Crowe, 1985), making this an important component of the frozen storage technique. In fact, considering that the only part of the procedure that the two earlier studies had in common was a warm, rapid thawing, it may be that slow thawing on ice is the most important and/or essential component for freeze–thaw vesicle preservation.

Mouradian *et al.* (1985) reported that, using trehalose as a protective agent, calcium uptake rates into sarcoplasmic reticulum (SR) remained constant for up to 100 days when these vesicles were lyophilized and stored under vacuum. In the present study, we used teleost intestinal BLMV to test the protective capabilities of two carbohydrates, mannitol and trehalose, when these vesicles were lyophilized and stored under vacuum. In addition, we tested the ability of trehalose to serve as a protective agent during lyophilization and vacuum storage of BBMV made from the same types of intestines. Other studies on SR membrane stabilization at low water activities have shown that mannitol may not be ideally suited to this preservation technique (Crowe *et al.* 1984*b*). Our data support these findings. The reduction in BLMV carrier-mediated D-glucose transport rates were mirrored rather closely by the reduction in equilibrium values, whereas there was only a small reduction in nonspecific binding. These results suggest that the reduction in transport rates in the nontrehalose-protected BLMV may be due to cracking, thus allowing dehydration-induced fusion upon rehydration (Womersley *et al.* 1986) and lateral phase transitions resulting in leakage through the bilayer and displacement of integral membrane proteins (Crowe *et al.* 1986, 1987), rather than actual damage to the transport carriers themselves. In contrast, the addition of trehalose to our BLMV and BBMV preserved the observed D-glucose carrier-transport processes. The superior protective ability of this carbohydrate has been suggested to be because the molecule, as a result of its size, is able to insert correctly between the phosphate headgroups of the bilayer (Womersley *et al.* 1986) and interact directly with them through hydrogen bonding (Crowe *et al.* 1984*b*), thus preventing lateral phase separations as water is removed.

This study extends to membranes derived from an epithelium the finding that the use of trehalose as the storage protective agent permits the preservation of membrane-bound carrier-transport characteristics during lyophilization, storage and rehydration of membrane preparations. Comparisons of BBMV and BLMV of vertebrate intestinal epithelium with sarcoplasmic reticulum show that epi-

thelial-derived vesicles are protected as well as dry SR preparations when excess trehalose is present.

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