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RESEARCH ARTICLE

Isolate and Characterise Brush Border Membrane Vesicles and Basolateral Membrane Vesicles from Equine Small Intestine

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Abstract

Membrane vesicles were isolated from the brush border membrane and basolateral membrane domains of equine enterocytes. In order to show that the isolated membrane vesicles derive either from the brush border or basolateral membranes and are pure, they were examined for the enrichment, dysenrichment of abundance, activity of specific marker proteins and enzymes characteristic of the brush border, basolateral and organelle membranes. Villin is a reliable marker of the intestinal brush border membrane. The result showed that there is a single immuno-reactive band for villin at 92.5 kilo Daltons in all 3 regions of the equine small intestine. This band is enriched in membrane vesicles over the homogenates and confirms that the membrane vesicles isolated do originate from the brush border membrane. The enrichment of alkaline phosphatase enzyme specific activity was increased (~ 6 to 11-fold) in BBMV over homogenates, further confirming that the vesicles were of brush border membrane origin. Na^+/K^+ ATPase is a classical marker protein of the intestinal basolateral membrane. The result showed a typical western blot for the levels of Na^+/K^+ ATPase in BLMV isolated from the small intestine of horse. The final pellet shows an enrichment of 95 and 40 kDa bands (α- and β- sub-units of the Na^+/K^+ ATPase), thus confirming that the vesicles are of basolateral membrane origin. Here the brush border membrane origin of vesicles has been assessed by showing enrichment of villin and alkaline phosphatase. Also the basolateral membrane origin of vesicles has been assessed by determining the enrichment of Na^+/K^+ ATPase in the basolateral membrane vesicles.

Introduction

Plasma membrane vesicles prepared from the lumenal and the basolateral membrane of enterocytes have proved to be important tools in describing the mechanisms underlying intestinal transport processes (5, 6, 10 and 16). In such preparations, the influence of cellular metabolism of metabolisable substrates such as glucose can be avoided. Furthermore, since the composition of intra and extra-vesicular media can easily be manipulated, the driving forces for the solute transport can be defined.

Transcellular transport of various nutrients, minerals and ions have been studied using vesiculated brush border and basolateral membranes (1, 5, 7, 10, 11, 16 and 20).B rush border membranes, when isolated by a sheering force, can only form right-side out vesicles, while basolateral membrane can form either right-side out or inside out vesicles. Presence of the glycocalyx on the lumenal membrane of the gut plasma membrane is a most important factor in formation of right-side out vesicles (2). Sidedness of the vesicles has been determined by freeze-fracture studies (3) and the latency of the marker enzyme activity (sucrase for the brush border membrane, and Na^+/K^+ ATPase for the basolateral membrane) in the absence and presence of a permeabilising agent such as detergents (4 and 8).

Material and methods

Animals and collection of tissue samples
Small intestinal tissues from 8 mature horses (aged 4-6 years as determined by dentition) maintained on a grass-based diet were collected from abattoir in Neston, Merseyside, UK within 15 minutes of slaughter. In order to ensure these horse' diet consisted of grass, the stomach and large colon was opened and any evidence of concentrate material resulted in the tissue being excluded. Sections of small intestine approximately 20 cm in length were removed from the duodenum (30 cm distal to the pylorus), jejunum (half way along the small intestine) and ileum (30 cm proximal to the ileo-caecal junction). They were opened longitudinally and rinsed in ice-cold buffered saline (0.9% (w/v) NaCl, pH 7.4) and then blotted with paper towels to remove excess mucous. The mucosa was removed by scraping, wrapped in aluminium foil and immediately frozen in liquid nitrogen. Following transportation to the laboratory, the frozen tissue samples were stored at -80ºC until use.

Brush Border Membrane Vesicles
Brush border membrane vesicles (BBMV) were isolated from equine small intestinal mucosal scrapings using a method based on that by Shirazi-Beechey *et al*. (11, 15 and 16). All steps were carried out at +4ºC.

Basolateral Membrane Vesicles
Basolateral membrane vesicles (BLMV) were isolated from equine small intestinal mucosal scrapings using a method based on that of Dyer *et al*. (5). All steps were carried out at +4ºC.

Estimation of Protein
Assay of protein concentration in the BBMVs and BLMVs was estimated using its ability to bind Coomassie Brilliant Blue G250 in acidic conditions according to the Bio-Rad assay technique. Bovine γ-globulin was used as the standard (6).

Enzyme assay
The activity of alkaline phosphatase was measured at 37ºC in cellular homogenates and BBMV as described by Dyer *et al.* (6).

Western blot analysis
The abundance of villin protein in the BBMV and Na^+/K^+ ATPase protein in the BLMV were determined by Western blotting as described previously (6 and 17). Protein components of BBMV and BLMV were separated by SDS-polyacrylamide gel electrophoresis on 8% (w/v) polyacrylamide mini-gels, containing 0.1% (w/v) SDS, and electrotransferred to PVDF membrane (Immun-Blot, Bio-Rad Laboratories Ltd., Hemel Hempstead, UK). For villin, the nitrocellulose membrane was blocked by incubating in block buffer (PBS / 5% (w/v) non-fat dried milk / 0.5% Triton X-100 / 0.1mM EDTA) for 1 hour with rocking at room temperature. For Na^+/K^+ ATPase, the nitrocellulose membrane was blocked by incubating in PBS-TM (PBS / 0.5% (w/v) non-fat dried milk / 0.05% Tween-20) for 1 hour with rocking at room temperature. The primary antibodies were diluted (1:2000 for villin) in wash buffer (PBS / 1% (w/v) non-fat dried milk / 0.5% Triton X-100 / 0.1mM EDTA) and (1:5000 for Na^+/K^+ ATPase) in PBS-TM and then the membrane incubated in these buffers for 1 hour with rocking at room temperature.
The membrane the washed 3 x 10 minute before incubation with HRP-conjugated goat anti-mouse secondary antibody for villin and swine anti-rabbit secondary antibody for Na^+/K^+ ATPase (DAKO) diluted 1:2000 in wash buffer and PBS-TM for 1 hour with rocking at room temperature, respectively. Before development using ECL (Amersham), the membrane was washed 3 x 10 minute as described previously (6).

Statistical Analysis
Statistical comparisons were made using one-way analysis of variance (ANOVA). P-values < 0.05 were considered significant and P-values < 0.01 were considered very significant.

Results

Brush border membrane vesicle isolation

Brush border membrane vesicles (BBMV) were prepared from equine small intestinal tissue samples from horses maintained on a conventional grass-based diet and their protein concentration measured as described in Methods. The protein content of BBMV used in this study was 20-30 mg/ml. The recovery and enrichment of protein from BBMV isolated from the small intestine (duodenum, jejunum and ileum) of several grass-fed horses showed in (Table 1).

	Recovery (%)	Enrichment (fold)
Duodenum	**5.27 ± 2.46**	**2.64 ± 1.23**
Jejunum	**4.48 ± 2.01**	**2.24 ± 1.00**
Ileum	**4.61 ± 1.90**	**2.31 ± 0.95**

Table1: Brush border membrane vesicle protein recovery and enrichment (n=8). Recovery is expressed as a percentage of vesicle total protein over homogenate total protein. Enrichment is expressed as a fold-increase of vesicle over homogenate concentration. Data is presented as the average ± standard deviation.

Characterisation of brush border membrane vesicles

i. Villin

Villin is a protein associated with the microfilament of the brush border membrane and is a reliable marker of the intestinal brush border membrane. Using a monoclonal antibody to villin, the enrichment of villin abundance in BBMV was used to determine the membrane origin of the isolated membrane vesicles. The result (Figure 1) showed a typical western blot of BBM isolated from the equine small intestine of grass-fed horses.

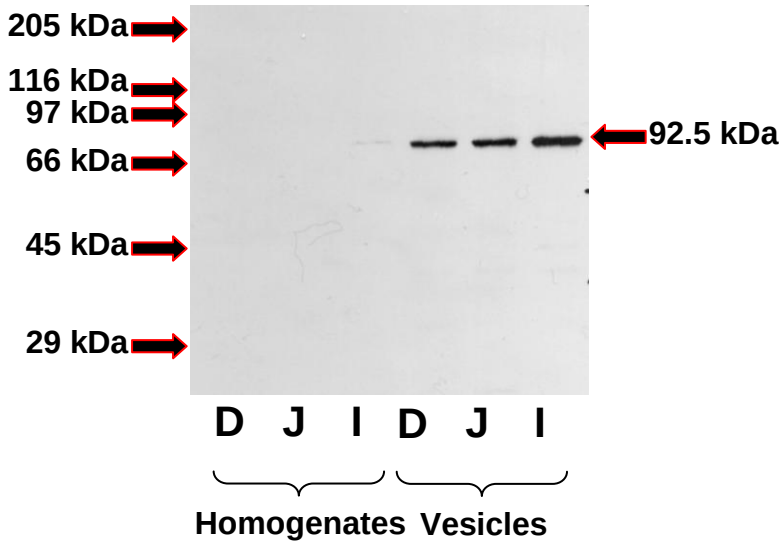

Figure 1: Western blot analysis of villin in homogenates and BBMV. Samples isolated from equine small intestine BBMV (20µg protein per lane) prepared from equine duodenum (D), jejunum (J) and ileum (I) were separated on polyacrylamide gels and electro-transferred to nitrocellulose or PVDF membranes as described in Methods. The figure showed a typical western blot for villin in homogenate and membrane vesicles, indicating the presence of a 92.5 kDa immuno-reactive band, and an enrichment of this band in vesicles over homogenates.

As can be seen from Figure 1, there is a single immuno-reactive band at 92.5 kilo Daltons in all 3 regions of the equine small intestine, consistent with the molecular weight of villin (9 and 13). This band is enriched in membrane vesicles over the homogenates and confirms that the membrane vesicles isolated do originate from the brush border membrane.

ii. Alkaline Phosphatase Activity

The activity of alkaline phosphatase, a brush-border membrane marker (17 and 18), was used to further determine the origin of membrane vesicles. The average specific activity (µmol/min/mg protein) and enrichment of Alkaline Phosphatase in homogenate and BBMV isolated from the 3 regions of the small intestine of 6 grass-fed horses showed in (Table 2).

Grass-fed horses	Alkaline Phosphatase Specific Activity (µmol/min/mg protein)						
	Homogenate	SEM	n	BBMV	SEM	n	Enrichment
Duodenum	0.142	0.044	5	1.599	0.270	6	11.26
Jejunum	0.162	0.057	5	1.843	0.360	6	11.38
Ileum	0.262	0.042	5	1.659	0.391	6	6.33

Table 2: Specific activity of alkaline phosphatase in grass-fed horses (n=6). Alkaline phosphatase activity was measured in homogenates and BBMV from the 3 regions of the equine small intestine as described in Methods. Enrichment is expressed as fold-increase of vesicles over homogenates.

The alkaline phosphatase activity (presented in Table 2) in the BBMV isolated from the grass-fed horses from the 3 regions of the equine small intestine is presented as a histogram in Figure 2. The enrichment of enzyme specific activity was increased (~ 6 to 11-fold) in BBMV over homogenates, further confirming that the vesicles were of brush border membrane origin. The levels of activity in the duodenum, jejunum and ileum were not significantly different (p = 0.838).

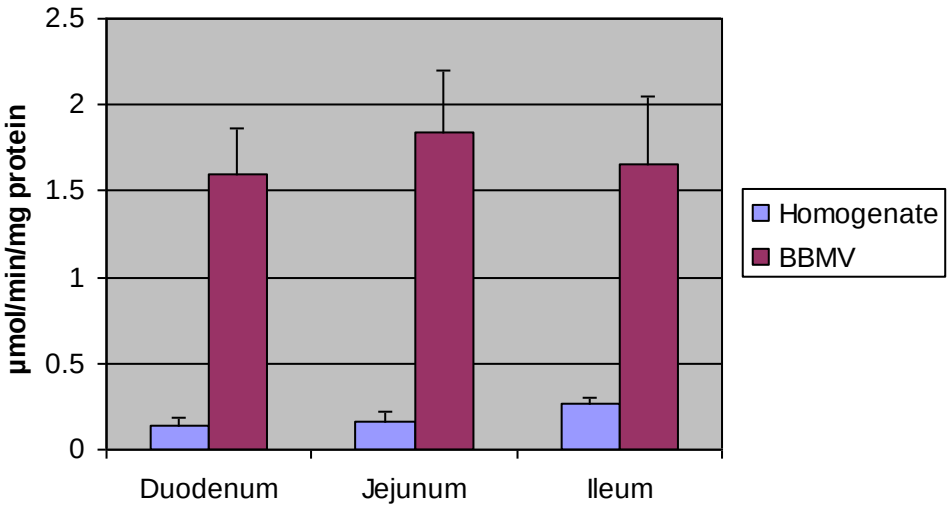

Figure 2: Alkaline phosphatase activity in horses (n=6). Histogram of the alkaline phosphatase specific activity taken from Table 2 showing levels of alkaline phosphatase activity in BBMV compared to cellular homogenate in the small intestine of grass-fed horses.

Basolateral membrane vesicle isolation

The recovery and enrichment of protein from basolateral membrane vesicles isolated from the equine small intestine (duodenum, jejunum and ileum) of several grass-fed horses showed in (Table 3). The protein content of BLMV used in this study was 20-30 mg/ml.

	Recovery (%)	Enrichment (fold)
Duodenum	**1.49 ± 1.19**	**3.73 ± 2.97**
Jejunum	**1.05 ± 0.78**	**2.62 ± 1.93**
Ileum	**1.43 ± 0.66**	**3.58 ± 1.63**

Table3: Basolateral membrane protein recovery and enrichment (n=6). Recovery is expressed as a percentage of membrane vesicle total protein over cellular homogenate total protein. Enrichment is expressed as a fold-increase of vesicle over homogenate concentration. Data is presented as the average ± standard deviation.

Characterisation of basolateral membrane vesicles

Na^+/K^+ ATPase

Na^+/K^+ ATPase is a classical marker protein of the intestinal basolateral membrane. The enrichment and abundance of Na^+/K^+ ATPase in the basolateral membrane vesicles was used to determine the membrane origin of the isolated membrane vesicles. The result showed a typical western blot of BLMV isolated from the small intestine of horse and sheep (Figure 3). Ovine BLMV were used as controls. The final pellet shows an enrichment of 95 and 40 kDa bands (α- and β- sub-units of the Na^+/K^+ ATPase), thus confirming that the vesicles are of basolateral membrane origin (4, 8, 14 and 19).

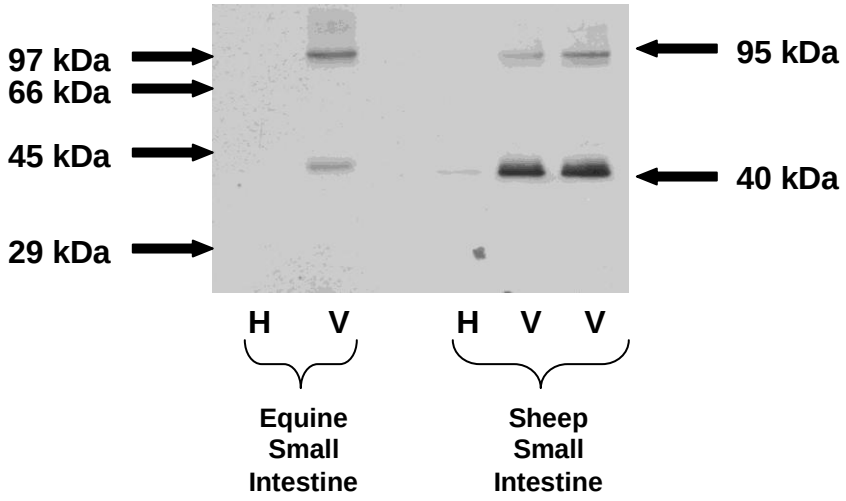

Figure3: Na^+/K^+ ATPase abundance in homogenates and BLMV isolated from equine small intestine. BLMV were prepared from equine and ovine jejunum. 20 µg of protein were loaded in each lane and separated on polyacrylamide gels and electro-transferred to nitrocellulose or PVDF membranes as described in Methods. The figure showed a typical western blot for the levels of Na^+/K^+ ATPase in homogenate and membrane vesicles, indicating the presence of a 95 and 40 kDa immuno-reactive bands, and an enrichment of this band in vesicles over homogenates. H = Homogenate, V = basolateral membrane vesicles.

Discussion

Brush border membrane vesicle confirmation

Using cation precipitation and differential centrifugation, it has been possible to isolate purified BBMV from the duodenum, jejunum and ileum of horses maintained on a conventional grass-based diet. The BBMV isolated by this procedure are devoid of any contamination by the basolateral and organelle membranes. This we have shown previously as enzymes, proteins characteristic of the basolateral and organelle membranes are not detected in the brush border membrane vesicle samples (6, 7, 15 and 16). Here the brush border membrane origin of vesicles has been assessed by showing enrichment of villin and alkaline phosphatase, which has previously been detected in equine small intestinal tissue samples (12).

Basolateral membrane vesicle confirmation

Using cation precipitation and density gradient centrifugation, it has been possible to isolate purified BLMV from the duodenum, jejunum and ileum of horses maintained on a conventional grass-based diet. We have shown previously that enzymes, proteins characteristic of the brush border and organelle membranes are not detected in the basolateral membrane vesicle samples prepared by this procedure (5 and 6). Here the basolateral membrane origin of vesicles has been assessed by determining the enrichment of Na^+/K^+-ATPase in the BLMV.

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Refrences

1. **Batchelor DJ**, Al-Rammahi M, Moran AW et al. (2011). Sodium/glucose cotransporter-1, sweet receptor and disaccharidase expression in the intestine of the domestic dog and cat: species of different dietary habit. *Am J Physiol Regul Integr Comp Physiol* 300, R67–R75.
2. **Bjorkman DJ**, Allan CH, Hagen SJ, Trier JS (1986). Structural features of absorptive cell and microvillus membrane preparations from rat small intestine, *Gastroenterology* 91: 1401-1414.
3. **Blitzer BL** and Donovan CB (1984). A new method for the rapid isolation of basolateral plasma membrane vesicles from rat liver. Characterization, validation, and bile acid transport studies, *J.Biol.Chem.* 259: 9295-9301.
4. **Boumendil-Podevin EF** and Podevin RA (1983). Isolation of basolateral and brush-border membranes from the rabbit kidney cortex. Vesicle integrity and membrane sidedness of the basolateral fraction, *Biochim.Biophys.Acta* 735: 86-94.
5. **Dyer J**, Beechey RB, Gorvel JP, Smith RT, Wootton R, Shirazi-Beechey SP (1990). Glycyl-L-proline transport in rabbit enterocyte basolateral-membrane vesicles, *Biochem.J.* 269: 565-571.
6. **Dyer J**, Fernandez-Castano ME, Salmon KS, Proudman CJ, Edwards GB, Shirazi-Beechey SP (2002). Molecular characterisation of carbohydrate digestion and absorption in equine small intestine, *Equine Vet.J.* 34: 349-358.
7. **Dyer J**, Al-Rammahi M, Waterfall L, Salmon KS, Geor RJ, Boure L, Edwards GB, Proudman CJ, Shirazi-Beechey SP (2009). Adaptive response of equine intestinal Na_/glucose co-transporter (SGLT1) to an increase in dietary soluble carbohydrate. *Pflügers Arch* 458: 419–430.
8. **Forbush B**, III (1982). Characterization of right-side-out membrane vesicles rich in (Na^+,K^+)-ATPase and isolated from dog kidney outer medulla, *J.Biol.Chem.* 257: 12678-12684.
9. **George SP**; Wang Y; Mathew S; Srinivasan K; Khurana S (2007). Dimerization and actin-bundling properties of villin and its role in the assembly of epithelial cell brush borders. *J. Biol. Chem.* 282:26528-26541.
10. **Kessler M**, Acuto O, Storelli C, Murer H, Muller M, Semenza G (1978). A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. Their use in investigating some properties of D-glucose and choline transport systems, *Biochim.Biophys.Acta* 506: 136-154.
11. **Moran AW**, Al-Rammahi M, Arora DK, Batchelor DJ, Coulter EA, Ionescu C, Bravo D, Shirazi-Beechey SP (2010). Expression of Na_/glucose co-transporter 1 (SGLT1) in the intestine of piglets weaned to different concentrations of dietary carbohydrate. *Br J Nutr* 104: 647–655.
12. **Roberts MC** (1974). The development and distribution of alkaline phosphatase activity in the small intestine of the horse. *Res.Vet.Sci.* 16: 110-111.
13. **Robine S**, Huet C, Moll R, Sahuquillo-Merino C, Coudrier E, Zweibaum A, Louvard D (1985). Can villin be used to identify malignant and undifferentiated normal digestive epithelial cells? *Proc.Natl.Acad.Sci.USA* 82: 8488-8492.
14. **Sheikh MI**, Kragh-Hansen U, Jorgensen KE, Roigaard-Petersen H (1982). An efficient method for the isolation and separation of basolateral-membrane and luminal-membrane vesicles from rabbit kidney cortex, *Biochem.J.* 208: 377-382.

15. **Shirazi-Beechey SP**, Gorvel JP, Beechey RB (1988). Phosphate transport in intestinal brush-border membrane, *J.Bioenerg.Biomembr.* 20: 273-288.
16. **Shirazi-Beechey SP**, Davies AG, Tebbutt K, Dyer J, Ellis A, Taylor CJ, Fairclough P, Beechey RB (1990). Preparation and properties of brush-border membrane vesicles from human small intestine, *Gastroenterology* 98: 676-685.
17. **Shirazi SP**, Beechey RB, Butterworth PJ (1981a). The use of potent inhibitors of alkaline phosphatase to investigate the role of the enzyme in intestinal transport of inorganic phosphate, *Biochem.J.* 194: 803-809.
18. **Shirazi SP**, Beechey RB, Butterworth PJ (1981b). Potent inhibition of membrane-bound rat intestinal alkaline phosphatase by a new series of phosphate analogues, *Biochem.J.* 194: 797-802.
19. **Wiener H**, Turnheim K, van Os CH (1989). Rabbit distal colon epithelium: I. Isolation and characterization of basolateral plasma membrane vesicles from surface and crypt cells, *J.Membr.Biol.* 110: 147-162.
20. **Wilson JW**, Webb KE, Jr. (1990). Simultaneous isolation and characterization of brush border and basolateral membrane vesicles from bovine small intestine, *J.Anim Sci.* 68: 583-590.