Mechanism of peptide transport in ruminant intestinal brush-border membrane.

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The absorption of peptides by the ruminant gastrointestinal tract is an important physiological process. The breakdown of proteins by rumen microorganisms gives rise to peptides, and recent observations suggest that these peptides make a significant contribution to non-ammonia nitrogen flow to the small intestine, and are available for absorption [1].

In the intestine of non-ruminant species the transport of di- and tri-peptides has been shown to be driven by a H⁺-gradient [2, 3, 4]. The H⁺/peptide symporter (PEPT1) has recently been cloned from rabbit intestine [5, 6] and may provide a useful tool in the identification of peptide transporters in other mammalian species.

The properties of a peptide transport system in the ovine and bovine enterocyte brush-border membrane were examined with glycyl-L-proline as the substrate.

Brush border membrane vesicles (BBMV) were isolated from ovine and bovine proximal intestine as described previously [7]. BBMV were fully characterised in terms of the marker enzymes characteristic of intestinal brush-border membrane. The membrane vesicles were enriched 23-fold in the brush-border membrane marker, alkaline phosphatase. The specific activities of marker enzymes associated with the basolateral membrane and intracellular membranes were low, indicating that there was little or no contamination of these membranes in the final BBMV preparation. The results were supported by Western blotting of membrane samples using antibodies raised against villin, a protein specific to cellular membranes were low, indicating that there was little or no contamination of these membranes in the final BBMV preparation. The results were supported by Western blotting of membrane samples using antibodies raised against villin, a protein specific to the pre-ruminant than in the proximal intestine as described previously [7].

The purified, fully characterised BBMV were then used to investigate the mechanism of transport of glycyl-L-proline in ruminant small intestine.

The ovine and bovine intestinal BBMV transported glycyl-L-proline in response to an inwardly-directed H⁺-gradient (pH<7.5, pH>7.5, see Fig. 1. Dissipating the H⁺-gradient, by pre-incubating the vesicles with 4,5,6,7-tetracloro-2-trifluoromethylbenzimidazole (TTFB), a classical mitochondrial uncoupling agent [9], inhibited the H⁺-dependent transport. The mechanism of H⁺/peptide transport, and the properties of the transporter in the intestine of ruminants, are similar to those observed in non-ruminant species [2, 3, 4]. The initial rates of glycyl-L-proline transport are higher in the pre-ruminant than in the ruminant animal.

The data initiates further work to investigate the molecular mechanisms regulating the expression of the peptide transporter at the cellular level.

![Graph showing peptide uptake](image-url)

**Fig. 1.** Glycyl-L-proline transport by ovine and bovine intestinal BBMV.

Glycyl-L-proline transport was measured as described previously [2]. BBMV (0.1 mg of protein), loaded with a buffer containing 300 mM-mannitol, 20 mM-Hepes/Tris, pH 7.5 and 0.1 mM MgSO₄, were incubated in 0.1 ml of a solution containing 100 mM KCl, 100 mM-mannitol, 1 mM-[^14]C]glycyl-L-proline, 0.1 mM MgSO₄ and either 20 mM-Hepes/Tris, pH 7.5, or 20 mM-Mes/Tris, pH 5.5. After 5 seconds the reaction was stopped by the addition of 1 ml of an ice-cold buffer containing 150 mM-KCl, 20 mM-Hepes/Tris, pH 7.5 and 0.1 mM-MgSO₄. A 0.9 ml sample of the stopped incubation mixture was then filtered through a 0.45 µm millipore cellulose nitrate/acetate filter, and the filter washed with 5 x 1 ml of the stop solution. The filter was then dissolved in 4 ml of scintillation fluid (Ecocine) and counted on a liquid scintillation counter (Beta V, Kontron). BBMV were also treated by incubation with TTFB (6.2 µg/mg protein), for 30 minutes, on ice, prior to the measurement of peptide uptake.