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H+/Di-tripeptide transporter (PepT1) expression in the rabbit intestine

Abstract In order to examine the intestinal expression of the recently cloned H+/di-tripeptide transporter (PepT1), oligonucleotide probes were synthesized and their specificity confirmed by Northern blot analysis of rabbit jejunal RNA. In situ hybridization studies, using these probes, show that PepT1 is expressed all along the small intestine and at a very much reduced level in the colon. In contrast, PepT1 mRNA was not detected in the stomach, sacculus rotundus or caecum. Microscopic examination of tissue sections showed PepT1 expression to be restricted to intestinal epithelium with no detectable expression in the lamina propria, muscularis mucosae, muscularis or serosa. The accumulation of PepT1 mRNA along the crypt-villus axis was also investigated. In all regions of the small intestine (in duodenum, jejunum and ileum), PepT1 mRNA was undetectable in deeper epithelial cells of the crypts. Expression was first detectable at or near the crypt-villus junction, the amount of PepT1 mRNA increasing rapidly in the lower villus to a maximum approximately 100–200 μm from this point. Along the length of the small intestine (in duodenum, jejunum and ileum), PepT1 mRNA was most abundant in duodenal and jejunal enterocytes, with lower levels in the ileal epithelium. PepT1 expression is greatly depressed in the follicle-associated epithelium of the Peyer’s patch relative to both interfollicular and adjacent “normal” villi. These data are discussed in the context of the known physiological role of PepT1 in the gastrointestinal tract.

Key words H+/Dipeptide transport · PepT1 · mRNA · Enterocytes · In situ hybridization

Introduction

The intestinal di-tripeptide transporter is an important membrane transport protein localized at the apical surface of the intestinal epithelium [6, 10] that plays a significant physiological role in the absorption of protein in the form of small peptides (2–3 amino acids in length). Unlike other ion/solute cotransporters of the gastrointestinal tract, transport via the di-tripeptide carrier is coupled to the movement of protons [10].

Measurements of intracellular pH taken during dipeptide transport across the apical membrane of intestinal cells indicate that the flux is directly coupled to the movement of protons [21]. In addition, the cotransport of di-tripeptides with protons is electrogenic both in membrane vesicles and in intact epithelial preparations [1, 10, 22]. The driving force for this H+-coupled carrier is thus the sum of the chemical activity gradient of protons across the brush-border membrane and the transapical membrane potential difference. The chemical activity gradient across the apical brush-border membrane may be of significance; an acid microclimate (an area of low pH lying adjacent to the apical surface of the intestinal epithelium) has been demonstrated in rat [14] and human jejunum [16]. Recently, the cDNA coding for a 707-amino acid peptide transporter (PepT1) was isolated from rabbit intestine using the Xenopus laevis expression cloning system [6]. When PepT1 cRNA was expressed in Xenopus oocytes there was a pH-stimulated transport of the non-hydrolysable dipeptide glycyl-sarcosine; transport being rheogenic and associated with acidification of the oocyte cytosol. High-stringency Northern blot analysis of poly(A)* RNA from rabbit tissues indicated a strong signal in the small intestine whereas the colon was negative. Weaker signals were also observed in kidney, liver and brain.

The purpose of the present study was to investigate the cellular expression of PepT1 along the adult rabbit gastrointestinal tract. Therefore, we developed two
oligonucleotide probes complementary to PepT1 mRNA for in situ hybridization studies.

Materials and methods

Materials were obtained from BDH, Poole, Dorset, UK unless otherwise stated.

Collection of tissue

Dutch rabbits (8 week old), which had been maintained on laboratory chow until used, were killed by an intravenous injection of a lethal dose of pentobarbitone (0.7 ml/kg, 20% w/v, Lethobarb, Duphar Veterinary, Southampton, UK). The entire small and large intestine was excised from the abdomen and washed with ice-cold phosphate-buffered saline (PBS) containing 0.5 mM dithiothreitol and 1U/ml RNase inhibitor (Sigma, Poole, Dorset, UK). The intestine was opened along the length of the mesenteric border. Samples of intestine were then rolled in a proximal to distal direction and intestinal preparations (“Swiss rolls”) were rapidly frozen in isopentane cooled with solid CO2. Samples were stored under liquid N2 until use.

In situ hybridization using oligonucleotide probes

The basic method used for in situ hybridization has been described previously [8]. Briefly, cryostat sections (10 μm) were thaw-mounted onto (poly) L-lysine coated slides and then fixed in 4% paraformaldehyde and stored in 95% ethanol at 4°C prior to in situ hybridization analysis. Anti-sense oligodeoxyribonucleotide probes complementary to the sequence of rabbit PepT1 cDNA [6] (see Table 1) were selected on the basis of their G-C content (55-65%) and lack of intra- and inter-probe complementarity. Probes were synthesized on a Biosearch 8700 DNA synthesizer and purified on an 8% polyacrylamide/8 M urea preparative sequencing gel. The sequences of the probes were unique to PepT1 when examined on the GenBank and EMBL databases. A random oligonucleotide probe (of 45 nucleotides) was used as a control for non-specific binding. Probes were labelled with [α-35S]dATP (>37 TBq/mmol, Amersham International, Amersham, Bucks, UK) using terminal deoxynucleotidyl transferase (Pharmacia, Milton Keynes, Bucks, UK) at 32 °C for 1 h. Probes were applied to the section in hybridization buffer containing 4× standard sodium citrate (SSC) and 50% deionized formamide. The slides were incubated overnight at 42 °C, before being washed in 1× SSC containing 0.1% sodium thiocyanate for 1 h at 55 °C and being dehydrated through alcohol.

Slides were exposed to Kodak Biomax X-ray film for 5 days, then dipped in Ilford K5 emulsion and stored, desiccated, at 4°C for 2 weeks. After development, sections were stained with eosin and mounted under a coverslip.

| Table 1 Sequence of anti-sense oligonucleotide probes (45 nucleotides) used for this study. Both anti-sense probes hybridized specifically to the gastrointestinal epithelium as described in the text. Probes taken from rabbit PepT1 cDNA sequence (Ac. No. U66467) [2, 6]. |
|---|---|---|
| Probe | Nucleotides | Sequence |
| PEPT1/1 | 1665–1709 | 5'- TGG CTC GTG ATC AGG TAC GTG TAC GCC CTG CCA AAC TCC AGG TAC -3' |
| PEPT1/2 | 776–820 | 5'- AGT CCA GCC AGT GCC CCC TCT TGG GAA ACT GCT TAC TGG GGT GCC -3' |

Quantification of in situ hybridization

The procedure used to quantify the in situ hybridization signal was similar to that used previously for the quantification of the sodium/glucose cotransporter (SGLT1) in rabbit small intestine [8]. Sections were examined using a MPV-3 microdensitometer (Leitz, Milton Keynes, Bucks, UK) at a magnification of ×400. The detection wavelength of the spectrophotometer was set at 640 nm. A measuring window (15 × 60 μm2) was placed over the lamina propria near to the villus to be measured and the absorbance reading was set at zero. Consistent absorbance readings were then taken from over the enterocyte population from the crypt base to the villus tip on five separate villi in the duodenum (5 cm from the pylorus), jejenum (50% along the small intestine) and ileum (5 cm from the ileal-caecal junction).

Northern blot analysis

Total tissue RNA from a sample of jejunum was extracted using the guanadidium thiocyanate method [4]. Total RNA (15 μg) was then separated on a formaldehyde denaturing 1.0% agarose gel by electrophoresis and blotted onto a Hybond N membrane (Amersham). RNA was fixed to the membrane by UV irradiation and molecular weight markers (BRL, Uxbridge, Middx, UK) were made visible by ethidium bromide staining. After prehybridization at 55°C for 4 h the blot was hybridized overnight at 55°C with a mixture of the two PepT1 anti-sense probes, 3′ tailed with [α-32P]dCTP in QuikHyb hybridization buffer (Stratagene, Cambridge, Cambbs, UK). After washing in 1× SSC at room temperature for 30 min and then twice at 55°C for 30 min, the blot was apposed to Kodak Biomax film for 2 days at −70°C in X-ray cassettes containing intensifying screens.

Analysis of data

The mean level of mRNA at each point along the crypt-villus axis was calculated from the mean of five readings from five separate villi selected from each sampling position. Since there were variations in the length of individual villi, mean absorbance values along the crypt-villus axis were included in the results only when over half the villi being measured still gave readings.

Results

Northern blot analysis

Northern blot analysis of total RNA extracted from rabbit intestine (Fig. 1) shows that the PepT1/1-2 anti-sense probes developed for this study hybridize to a

![Fig. 1 Northern blot analysis of anti-sense probes for the H+/dipeptide transporter (PepT1). Hybridization to total RNA (15 μg/lane) extracted from rabbit small intestine was performed under similar conditions of stringency as the in situ hybridization. Specificity is confirmed by the identification of a 2.9 kb band comparable to that observed by Fei et al. [6]](image-url)