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Levels of disaccharidases in the brush border membrane of equine small intestine

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Abstract

The disaccharides, consisting of sucrose, lactose and maltose, are hydrolysed into monosaccharides (D-glucose, D-galactose and D-fructose) by intestinal brush border enzymes: sucrase, lactase and maltase. The aim of this study to investigate changes in the brush-border membrane carbohydrate digestive enzymes. From intestinal mucosal scrapings of equine, brush border membrane vesicles were isolated. The results showed that sucrase, maltase and lactase are present in the equine small intestine. The activity of all three enzymes is highest proximally (in the duodenum and jejunum) and lower in the ileum. There was considerable variation between individual horses, however the majority showed highest disaccharidase activity in the jejunum, with some showing highest activity in the duodenum. Sucrase activity is highest in the jejunum and duodenum and lower in the ileum. Maltase activity is similar in all three regions, but slightly higher in the jejunum. Lactase activity is low in all three regions of the small intestine, slightly higher in the equine jejunum and duodenum than ileum. From this study, we can conclude that the equine small intestine digests disaccharides by the brush-border associated disaccharidases sucrase, maltase and lactase. Levels of sucrase and lactase are comparable to other species, but maltase is much higher.

***Keywords*:** Brush border membrane vesicle, Sucrase, Maltase, Lactase, Equine small intestine
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مستويات إنزيمات السكريات الثنائية في الغشاء الحافي الفرشاتي لأمعاء الخيول

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الخلاصة

السكريات الثنائية وهي السكروز، اللاكتوز والمالتوز تتحلل الى سكريات أحادية (دي-كلوكوز، دي-كالكتوز ودي-فركتوز) بواسطة أنزيمات الغشاء المعوي الفرشاتي وهي السوكريز، اللاكتيز والمالتيز. الهدف من الدراسة هو التحري عن التغيرات في الأنزيمات الهضمية المعوية الموجودة في الغشاء الفرشاتي للامعاء. تم عزل حويصلات الغشاء الفرشاتي من طبقة الغشاء المخاطي المقشوطة من الأمعاء الدقيقة للخيول. أظهرت النتائج أن انزيمات السوكريز، المالتيز واللاكتيز موجودة في الأمعاء الدقيقة للخيول. حيث بينت النتائج أن نشاط الإنزيمات الثلاثة أعلى تقريباً (في الأثنى عشر والصائم) وأقل فعالية في اللفائفي. كان هناك تباين واضح بين الخيول إلا أن الغالبية أظهرت اعلى نشاط للأنزيمات في الصائم كذلك أظهر البعض نشاطاً أعلى في الأثنا عشر. حيث بينت النتائج بأن فعالية أنزيم السوكريز تكون أعلى في الصائم والإثنى عشر واقل فعالية في اللفائفي بينما كانت فعالية أنزيم المالتيز متساوية في كل من الاثنى عشر، الصائم واللفائفي ولكنها أعلى قليلاً في الصائم. بينما كانت فعالية أنزيم اللاكتيز منخفضة في كل أجزاء الأمعاء الدقيقة ولكنها أعلى قليلاً في الصائم والاثنى عشر مقارنة باللفائفي. من خلال هذه الدراسة، يمكننا أن نستنتج بأن الأمعاء الدقيقة في الخيول تهضم السكريات الثنائية بواسطة ألانزيمات الثنائية (السوكريز، المالتيز واللاكتيز) المرتبطة بالغشاء الفرشاتي وأن مستويات الأنزيمات الثنائية السوكريز والمالتيز مماثلة للأنواع الأخرى ولكن مستوى أنزيم المالتيز أعلى بكثير.

Introduction

In non-ruminant herbivore species, such as horse, the natural diet is grass from pasture forage. The large intestine (caecum and colon) of horses is an immensely enlarged fermentative chamber which contains a uniquely adapted microbial population (1-3). The microbial fermentation of dietary plant fibre leads to the production of monocarboxylates, acetate, propionate and butyrate, often referred to as short chain fatty acids (SCFAs). The absorption of these SCFAs via the colonic epithelium provides a significant proportion of the horse's energy requirements (1-3). Today's horses are normally fed more concentrate-based diets containing high levels of digestible carbohydrates (2). It is therefore important that in order to prevent intestinal dysfunction, horses are able to digest these carbohydrates before they enter the large intestine (3). It has been shown in many species that digestible carbohydrates are hydrolysed into their component monosaccharides in the lumen of small intestine by pancreatic α-amylase and the brush border membrane disaccharidases (sucrase, maltase and lactase) (4,5). Sucrase hydrolyses sucrose into glucose and fructose, lactase hydrolyses lactose into glucose and galactose, and maltase hydrolyses maltose into two molecules of glucose (6). Glucose and galactose are then transported across the brush border membrane (BBM) of intestinal enterocytes via the sodium-dependant glucose transporter SGLT1 (7-9), while fructose is absorbed into the enterocyte via the sodium-independent fructose transporter GLUT5 (9-11). These monosaccharides are then transported into systemic circulation across the basolateral membrane of the intestinal enterocyte via the sodium-independent monosaccharide transporter, GLUT2 (4,5,8,10).

The aim of this study is to investigate expression of brush-border membrane carbohydrate digestive enzymes in the equine small intestine.

Material and methods

Animals and collection of tissue samples

Intestinal samples from duodenum, jejunum and ileum from 7 mature horses aged 4-6 years were collected from a local abattoir in Neston, UK and treated as described by Al-Rammahi (12).

Brush border membrane vesicles

From equine small intestinal mucosal scrapings, brush border membrane vesicles (BBMV) were isolated using a method based on that by (13) as described (3,12). The final pellet which containing purified BBMV was homogenized by passing through a 27 gauge needle several times in 0.1 mM $MgSO_4$, 300 mM mannitol, 0.02% (w/v) NaN_3 and 20 mM HEPES/Tris, pH 7.4 buffer. Until used, the BBMV were aliquoted and stored in liquid nitrogen. All steps were carried out at $^{+}4^{o}C$ (5,12).

Estimation of protein

According to the Bio-Rad assay technique, the protein concentration was estimated in the BBMVs using its ability to bind Coomassie Brilliant Blue G250 in acidic conditions. We used the bovine γ-globulin as a standard (12,14,15).

Disaccharidase activity

In a glass test-tube, 50µl of BBMV was placed (in triplicate). The samples were placed in a water bath at 37 ºC and allowed to reach temperature as described previously (2,3,14). The reaction was started by the addition of 50 µl assay mix (depending on which enzyme was being assayed); Sucrase - 100 mM NaH Maleate pH 6.0, 56mM Sucrose; Lactase - 100 mM NaH Maleate pH 6.0, 56 mM Lactose, 200 mM para-Chloromercuric Benzoate (PCMB) and Maltase - 100 mM NaH Maleate pH 6.0, 56 mM Maltose. Then incubated at 37 ºC for 15 minutes. The reactions were stopped by placing the tubes in a boiling water bath at 100 ºC for 2 minutes and then cooling them to room temperature. 1 ml of Solution 1 (Boehringer Mannheim; containing Triethanolamine buffer pH 7.6, NADP, ATP and $MgSO_4$) and 1.9 ml double distilled H_2O were added to each tube and the solution mixed. Finally, 20 µl of Suspension 2 (Boehringer Mannheim; containing hexokinase, glucose-6-phosphate dehydrogenase) were added to each tube and the reaction mixed and allowed to stand for 15 minutes. Afterwards aliquots were placed in 1 ml cuvettes and the absorbance of 1 cm path length at 340 nm was measured (U-2000 Spectrophotometer, Hitachi).

Statistical Analysis

Data are presented as means ± SEM. Significance statistical comparisons were determined by using one-way analysis of variance (ANOVA). Results were considered significant when P values < 0.05.

Results

Sucrase

The enrichment (6.6-10.6 fold) of sucrase activity in vesicles over homogenate confirmed that the membrane vesicles isolated were of BBM. The results showed that sucrase specific activity (µmol/min/mg protein) in the 3 regions of the small intestine of 7 grass-fed horses is highest in the proximal small intestine (duodenum and jejunum) compared to the distal (ileum) (table 1), and this data is represented as a histogram in figure 1.

Maltase

The results showed that maltase activity (µmol/min/mg protein) of 7 grass-fed horses is similar in all regions of the

small intestine table 2, and this data is represented as a histogram in figure 2.

Lactase

The finding showed that the lactase specific activity (µmol/min/mg protein) in 3 regions of the small intestine of 7 grass-fed horses is highest in the proximal small intestine (jejunum > duodenum) compared to the distal (ileum). Table 3 shows the lactase specific activity, and this data is presented as a histogram in figure 3.

Table 1: Specific activity of sucrase

	Sucrase Specific Activity (µmol/min/mg protein)						
	Homogenate	SEM	n	Vesicles	SEM	n	Enrichment
Duodenum	0.036	0.006	7	0.380^{ns}	0.078	7	10.6
Jejunum	0.042	0.010	7	0.395*	0.075	7	9.4
Ileum	0.033	0.004	7	0.216*	0.029	7	6.6

Enrichment is fold-increase of sucrase specific activity in BBMV over homogenate, * *P* <0.05, ns not significant.

Table 2: Specific activity of maltase

	Sucrase Specific Activity (µmol/min/mg protein)						
	Homogenate	SEM	n	Vesicles	SEM	n	Enrichment
Duodenum	0.059	0.008	7	0.831^{ns}	0.113	7	14.1
Jejunum	0.075	0.016	7	0.908^{ns}	0.106	7	12.1
Ileum	0.086	0.011	7	0.776^{ns}	0.111	7	9.1

Enrichment is fold-increase of maltase specific activity in BBMV over cellular homogenates, ns not significant.

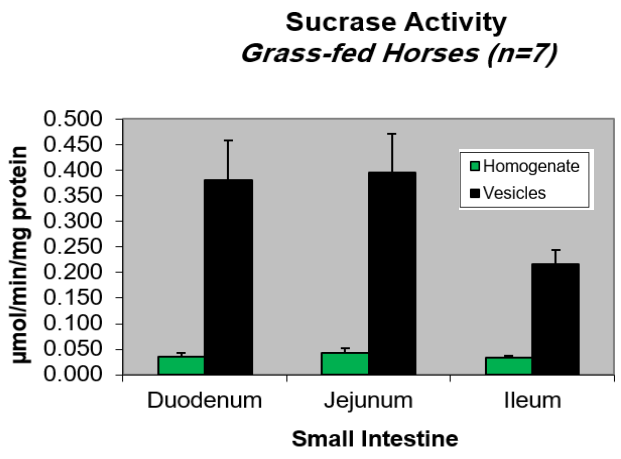

Figure 1: Sucrase specific activity in all 3 regions of the equine small intestine showing the enrichment in BBMV over cellular homogenates.

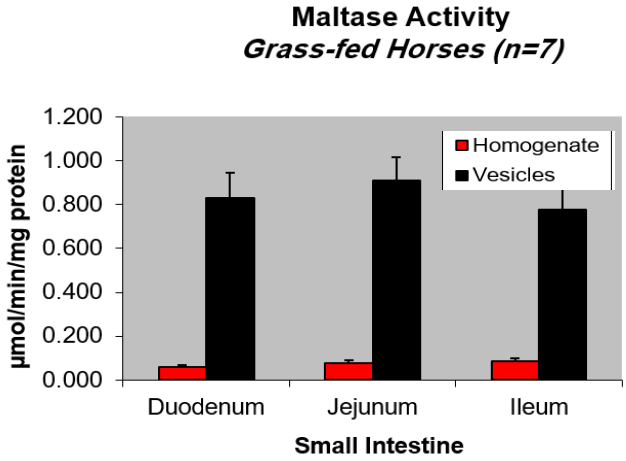

Figure 2: Maltase specific activity in all 3 regions of the equine small intestine showing the enrichment of in BBMV over cellular homogenates.

Table 3: Specific activity of lactase

	Sucrase Specific Activity (µmol/min/mg protein)						
	Homogenate	SEM	n	Vesicles	SEM	n	Enrichment
Duodenum	0.030	0.004	7	0.082^{ns}	0.024	7	2.7
Jejunum	0.029	0.005	7	0.100^{ns}	0.030	7	3.4
Ileum	0.025	0.005	7	0.047^{ns}	0.006	7	1.9

Enrichment is fold-increase of maltase specific activity in BBMV over cellular homogenates, ns not significant.

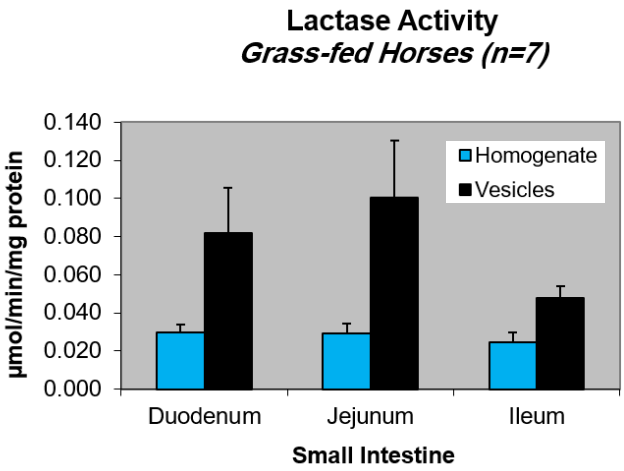

Figure 3: Histogram of lactase specific activity in all 3 regions of the equine small intestine showing the enrichment in vesicles over cellular homogenates.

Discussion

The horses' natural diet, grass from pasture forage, undergoes seasonal variation in its soluble (hydrolysable) carbohydrate content (16). However, this variation is much less than in the natural diet of most omnivores, and is certainly less than the difference between grass and the high grain (starch) concentrate diets fed to many horses in managed environments (3). It has previously been proposed that the equine intestine may have a slower or blunted adaptive response to dietary change (17), which is be an important consideration for the development of dietary-induced intestinal dysfunction in horses. It is not known however if the slower adaptive response is in the digestion or the absorption of dietary carbohydrates. Starch is mainly hydrolysed in the small intestine by pancreatic α-amylase and the intestinal brush-border membrane disaccharidase, maltase, to glucose (18,19).

We can see here that the activity of maltase in the equine jejunum (0.908 µmol/min/mg) is up to 3 times higher than that in other species such as pig (0.306 µmol/min/mg) and cat (0.400 µmol/min/mg) (2) and it is therefore unlikely that there is a deficiency in maltase activity limiting starch digestion in the horse (20,21). However, it has been shown that the levels and activity of α-amylase in the equine intestine are low compared to other species (4,5), and it would seem probable that the initial breakdown of starch into maltose, maltotriose, and α-dextrin is the limiting step of starch digestion in horses given concentrate diets containing large amounts of grain (22).

In this study, we compared the activity of disaccharidases along the length of the small intestine of horses maintained on grass. In the horse small intestine, SGLT1 is only slowly upregulated over time, a lag period which may be due to the time required for α-amylase upregulation and greater rates of starch hydrolysis (3). Studies carried out in other species have indicated that there is an adaptive response in amylase expression and activity in response to increases in hydrolysable dietary carbohydrates (23). This is also true in horse, however, the rate of increase is much smaller than in omnivorous species such as pigs or even in the carno-omnivorous dog Kienzle *et al* (21) suggesting that a longer term adaptation period is needed for enhancement in α-amylase activity in response to increased dietary hCHO in horse (22). Increased knowledge of the digestive system of the horse, and insight into the underlying mechanisms of intestinal adaptation in response to a change in diet, will help to improve the development of scientifically-based dietary strategies that could be used to modify the capacity of the equine intestine to digest hydrolysable carbohydrates with the aim of enhancing feed formulation and improving the health and welfare of the horse.

Conclusion

From these results it is clear that the equine small intestine is expressing disaccharidases in the brush border membrane. The equine small intestine is capable of digesting disaccharides by the brush-border associated disaccharidases sucrase, maltase and lactase. Levels of sucrase and lactase are comparable to other species, but maltase is much higher.

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