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THE PALMITIC ACID BINDING PROPERTIES OF CYTOSOLIC PROTEINS LOCATED IN THE VILLUS AND CRYPT ZONES OF BOVINE INTESTINAL MUCOSA

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ABSTRACT

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The palmitic acid binding capacity of cytosolic proteins in three preparations obtained by differential scraping of bovine intestinal mucosa were compared. The data indicated that the palmitic acid binding activities depended on the position that the cells occupied along the crypt–villus axis, as shown from the level of alkaline phosphatase activity. Proteins with palmitate binding properties in the high- and low-molecular-weight cytosolic proteins in the villus zone bound 1.24 ± 0.41 and 1.54 ± 0.16 pmol palminate/μg protein respectively. The binding decreased to 0.50 ± 0.25 and 1.10 ± 0.23 pmol palmitate/μg for the proteins in the crypt zone. Ammonium sulphate fractionation and gel filtration chromatography indicated that the low-molecular-weight cytosolic proteins obtained from light mucosal scrapings contained the highest palmitate binding activity. These results suggest that the cytosolic proteins located in the villus zone may play a role in the absorption of fatty acids.

Keywords: alkaline phosphatase, cattle, intestine, mucosa, palmitic acid, protein

INTRODUCTION

Although considerable progress has been made in the study of long-chain fatty acid absorption (Ockner *et al.*, 1972), little is known concerning the transport of fatty acids across the bovine intestinal mucosa (Noble, 1981). The mechanism of fat absorption, re-esterification, packaging into chylomicrons and their exit via the lateral membrane is unique to the enterocyte (Simmonds, 1976). This cell has well-defined histological domains that appear to be functionally different. Active transport of monosaccharides and amino acids appears to be a property of the microvillus membrane of the differentiated villus cell (Garvey *et al.*, 1976). Active ion and water secretion has been attributed to the crypt zone. Since binding to the plasma membranes of the different domains is the first step in the cellular uptake mechanism, followed by the passage of the compound through the cytoplasm, a study was designed to characterize the proteins that bind palmitic acid and are located in the cytosol of the villus and crypt zones of the bovine intestinal mucosa.

MATERIALS AND METHODS

[1-^{14}C]Palmitic acid, 58 mCi/mmol, was provided by New England Nuclear, Boston, USA. Sephadex G75 was obtained from Pharmacia Fine Chemicals, Inc., Piscataway, NJ, USA. Disodium 4-nitrophenylphosphate hexahydrate was obtained from Fluka Chemie AG, Buchs, Switzerlasnd Lipidex 1000 came from Packard Instrument Company, Inc., Drowners Grove, IL, USA.

Preparation of the bovine intestinal mucosal scrapings

Segments of small intestine were removed from mature cows 15 min after death and maintained at 4°C. The intestines were opened longitudinally and flushed with 0.15 mol/L NaCl; the adherent fat tissue was removed and the mucosa was scraped off. Material from different depths was recovered by applying light, moderate and strong pressure respectively by hand with a glass slide, giving the superficial, middle and deep fractions respectively.

Cytosol preparation

Each mucosal scraping was suspended in 15 ml of a solution containing 0.25 mol/L Tris–HCl, pH 7.4, with 0.01% w/v NaN_3 (solution A) and homogenized with a glass–Teflon homogenizer. Aliquots were preserved at −22°C for later determination of alkaline phosphatase and protein. The cytosols obtained from the homogenates were subjected to gel filtration on Sephadex G75 in order to obtain two large fractions F_1 and F_2. F_1 corresponded to proteins with molecular weights greater than 68 kDa and F_2 represented proteins in the range 12–16 kDa (Palacios and Catalá, 1989).

Alkaline phosphatase activity

This was assayed using a reaction mixture containing 10 mmol/L *p*-nitrophenol phosphate and 0.5 mmol/L Mg^{2+} in 50 mmol/L carbonate–bicarbonate buffer, pH 9.5. Each sample of mucosal scraping (5–20 μg of protein) was incubated at 35°C in 1 ml of the reaction mixture for 20 min. The reaction was terminated with 5 ml of 5 mmol/L EDTA in 0.02 mol/L NaOH and the amount of *p*-nitrophenol released was estimated by measuring the absorbance at 405 nm. The enzyme activity was expressed in U/mg protein, with each unit equivalent to 1 μmol substrate transformed per min.

Other methods

Partial purification of fatty acid binding protein by ammonium sulphate fractionation was done as described by Avanzati and Catalá (1983). Protein was determined by the method of Lowry *et al.* (1951) using bovine serum albumin as the standard. Palmitate binding by soluble proteins was assessed by the lipidex assay technique described by Glatz and Veerkamp (1983).

RESULTS

In initial experiments we examined the alkaline phosphatase activity of the whole homogenates prepared by differential scraping of bovine intestinal mucosa. The highest alkaline phosphatase activity was associated with the superficial and middle fractions (Table I), whereas there was only low activity in the deep fraction. The protein in the superficial scraping, which represented 50.47% of that present in the whole mucosa, possessed two cytosolic protein fractions with palmitic acid binding capacity as demonstrated by gel filtration on Sephadex G75 (Palacios and Catalá, 1989). The low-molecular-weight protein fraction (F_2) was more effective than the high-molecular-weight protein fraction (F_1) in binding palmitic acid using the lipidex assay. The results in Table I also indicate that both the F_1 and F_2 obtained by the light or moderate scraping had greater fatty acid binding capacity than the similar fractions obtained from the deep scraping. When the cytosols were fractionated with 70% saturated ammonium sulphate, the amount of protein in solution was greatest in the superficial and lowest in the deep scrapings (Table II). The lipidex assay showed that, of the proteins that remained in solution after ammonium sulphate precipitation, those in the deep scraping had least palmitic acid binding activity (Table II). When the proteins which were soluble in 70% ammonium sulphate were fractionated by Sephadex G75 gel filtration, the bulk of the protein eluted in the low-molecular-weight fraction. The greatest binding of palmitate to an F_2 fraction was with the material obtained from the superficial scraping. The high value of palmitate binding to F_1 from the deep fraction may have been due to albumin contamination (Table III).

TABLE I

The protein content and the alkaline phosphatase and palmitic acid binding activities of the cytosolic proteins obtained from different depths in the bovine intestinal mucosa

Scraping	Protein (mg)[a]	Alkaline phosphatase activity (U/mg protein)	Palmitic acid binding activity (pmol palmitate/μg protein)	
			F_1[b]	F_2[b]
Superficial	464 336	0.51 ± 0.04	1.24 ± 0.41	1.54 ± 0.16
Middle	267 204	0.33 ± 0.04	1.63 ± 0.40	1.85 ± 0.13
Deep	177 135	0.008 ± 0.001	0.50 ± 0.25	1.10 ± 0.23

[a]The amounts of protein scraped from 1 m of intestine of two different animals

[b]F_1 (>768 kDa) and F_2 (12–16 kDa) were obtained as described by Palacios and Catalá (1989)

Values are mean ± SEM of three determinations

TABLE II
The palmitate binding activity of the soluble protein obtained by ammonium sulphate fractionation of cytosol from bovine intestinal mucosa

Scraping	Soluble protein (g/L cytosol)[a]	Palmitate binding activity (pmol palmitate/μg protein)
Superficial	3.0	1.9 ± 0.4
Middle	1.4	1.8 ± 0.3
Deep	0.88	0.5 ± 0.1

[a]The protein that remained in solution after precipitation with 70% ammonium sulphate precipitation, expressed as g/L of cytosol

TABLE III
The palmitate binding activity of the protein fractions which were soluble in 70% ammonium sulphate and those separated with Sephadex G75

Scraping	Proteins (%)[a]		Palmitate binding activity (pmol palmitate/μg protein)	
	F_1[b]	F_2[b]	F_1[b]	F_2[b]
Superficial	19.0	81.0	0.3 ± 0.05	4.7 ± 0.2
Middle	34.0	66.0	0.5 ± 0.0	0.7 ± 0.2
Deep	3.0	97.0	5.9 ± 1.3	2.1 ± 0.6

[a]Protein content as a percentage of the total amount of protein eluted from the column
[b]F_1 and F_2 were the fractions obtained, using Sephadex G75, of >68 kDa and 12–16 kDa respectively

DISCUSSION

The mechanism by which fatty acids are absorbed by the bovine intestinal mucosa is unclear. For many years the cellular uptake of fatty acids was considered to be a passive, diffusional process; only recently has the presence of a fatty acid binding protein in the apical and lateral portions of the brush border cells of the jejunum, but not on the luminal surface of oesophagus or colon, been shown (Stremmel *et al.*, 1985). Cytosolic fatty acid binding proteins have been described in the intestine of both the pre-ruminant calf (Jenkins, 1985) and the mature bovine animal (Palacios

and Catalá, 1989). However, there is insufficient knowledge of the mechanisms by which long-chain fatty acids are absorbed by intestinal cells in ruminants.

Our results clearly indicate that the cytosol obtained from mucosal scrapings of bovine intestine contains two protein fractions, of high and low molecular weight respectively, with palmitic acid binding properties. The data suggest that the binding capacity of these proteins depends on the position they occupy along the villus–crypt axis, as indicated from the alkaline phosphatase activity in the homogenates from which they were obtained. The high alkaline phosphatase activity in the mucosal scrapings obtained by the application of light pressure is a clear indication of enrichment with villus cells (Hartman *et al.*, 1982). The progressive decrease in the binding of palmitic acid to cytosolic proteins from the superficial to the deep fractions therefore suggests that the transport of palmitic acid at the intracellular level is a property of the villus cell. In this regard it is important to note that it has already been demonstrated (Stremmel, 1988) that the uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding protein located in the brush border membrane. Ockner and Manning (1974) studied the *in vivo* binding of [^{3}H]palmitic acid to rat intestinal fatty acid binding protein (FABP). They demonstrated that more radioactivity became bound to the villi than to the crypts. Of the radioactivity associated with the supernatant from the villus, 20% was associated with FABP, whereas only 9% of that in crypts was so bound. In a recent paper, Iseki *et al.* (1989) demonstrated that FABP immunoreactivity is confined to the absorptive cells of the villi and is not associated with the crypts in the small intestine of rats fed *ad libitum*.

Our findings may be interpreted as suggesting that the palmitic acid binding proteins located in the villus zone of bovine intestinal mucosa play an important role in intracellular fatty acid transport or utilization.

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