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Acylase 1 expression in rat intestinal crypt–villus axis

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Abstract

Acylase 1 was investigated at the cellular level in the rat small intestine along the enterocyte–differentiation axis. As confirmed by microscopic analysis, villus tip cells and crypt cells of rat jejunal mucosa were successfully separated using the Weiser method. The proliferating undifferentiated crypt cells showed much higher ACY 1 activity levels than the villus cells, with a 6.4-fold decrease as the cells migrated and differentiated along the crypt–villus axis. RT-PCR studies on mRNA extracted from isolated cells showed that ACY 1 mRNA was mainly expressed in crypt cells, reaching levels that were 12-fold higher than those recorded in other cell types along the whole enterocyte differentiation axis. It was concluded that the expression of ACY 1 in the intestinal crypt cells is regulated at the mRNA level. Immunohistochemistry revealed the expression of ACY 1 in the absorbing lineage cells from the ileal and colonic crypts and the absence of ACY 1 in the mucus producing goblet cells. These findings proposed ACY 1 as a new marker transcript for absorbing cells of intestinal crypt, which can be used to monitor the process of intestinal *N*-α-acetylated protein metabolism.

Keywords: Acylase 1; N-terminal acetylated protein; Enterocyte differentiation; Weiser method

1. Introduction

Enterocytes constitute the bulk of the epithelial population, which is mainly responsible for the absorptive process occurring after the action of specialized brush border hydrolases has been completed. Mammalian small intestinal epithelial cell production begins with crypt cells dividing and subsequently differentiating along the crypt–villus structural axis. Highly regulated mechanisms control the rate of proliferation and differentiation of enterocytes as they move along the crypt–villus axis during their relatively short lifetime (48–72 h in the rat) (De Santa et al., 2003; Vidrich et al., 2003). The process of mitosis ends and that of differentiation starts in the crypt. Differentiation is completed in the lower half of the villus, and there exists histological evidence that apoptosis occurs at the villus tip (Potten and Loeffler, 1987; Madara and Trier, 1987; Weiser, 1973; Altmann and Leblond, 1982; Gravieli et al., 1992).

During differentiation of the mouse small intestinal epithelium, a general decrease in transcriptome complexity, using filter-based cDNA arrays, was demonstrated (Tadjali et al., 2002). The marked polarization of this cell, as well as the expression of hydrolytic brush border enzymes, provide specific markers which can be used to monitor the process of enterocyte differentiation (Weiser, 1973; Tadjali et al., 2002; Traber, 1990; Noren et al., 1989; Darmoul et al., 1991). Several studies have also shown that a few xenobiotic-metabolizing enzymes are present in the fully differentiated enterocytes (Traber et al., 1992; Cornell and Meister, 1976; Ware et al., 1998).

Abbreviations: Ac, acetyl; ACY 1, acylase 1.

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An interesting online intestinal crypt/villus *in situ* hybridization database holds information for relative gene expression patterns in the mammalian intestine (Olsen et al., 2004). In mouse small intestine, a high expression of cell cycle and DNA synthesis genes occurs in the crypt, and cytoskeletal and lipid uptake genes are present in the villus, in respect with the enterocyte differentiation (Mariadson et al., 2005).

In mammalian cells, as much as 70% of the cytosolic proteins can be α-*N*-acetylated, and the *N*-protected amino acids which are released during proteolysis must be free of amino acids before they can be used for protein synthesis (Driessen et al., 1985), since it seems hardly likely that they would be wasted in the cells. Post-translational acetylation of intracellular proteins and peptides is generally thought to be an efficient mechanism protecting them from proteolytic degradation in eukaryotic cells (Persson et al., 1985; Tsunasawa and Sakiyama, 1984). The NH_2-α-acetylated amino acid released from the protein or the resulting peptide is catalyzed by the sequential action of the acylpeptide hydrolase (APH or acylamino acid releasing enzyme, EC 3.4.19.1) and acylases (ACY, EC 3.5.1.x) generating a free amino acid and an acetate, yielding the corresponding free amino acid during the process of intracellular protein catabolism.

Acylase 1 (ACY 1 or *N*-α-acyl-L-amino acidamidohydrolase, EC 3.5.1.14) is by far the best characterized form, which hydrolyses aliphatic *N*-acyl-α-amino acids in tissue extracts (Gade and Brown, 1981; Perrier et al., 2005). It is worth noting here that the α-*N*-acetylamino acids which are usually found at the N-terminal position of the acylated protein, namely Ac-methionine, Ac-alanine and Ac-serine (Driessen et al., 1985), correspond to the substrate specificity of ACY 1. The free amino acid released, especially the essential amino acid methionine (Met), is required for initiating protein synthesis by Met-tRNA formation, in relation to the polyamine synthesis or the *trans*-methylation pathway. Alternatively, polyamine synthesis abets normal cell proliferation (Ogier et al., 1993).

However, until now, the exact physiological function of ACY 1 has not yet been completely elucidated. The fact that high levels of this enzyme have been detected in various tissues, including intestinal epithelia (Giardina et al., 1997), strongly suggested that this enzyme might have some additional physiological functions. For example, the expression of ACY 1 has been reported to be correlated with the existence and activity of acetylated growth factors in carcinoma cells and to be down-regulated in these cells (Scaloni et al., 1992; Hwa et al., 2005). ACY 1 might also regulate the activity of type 1 sphingosine kinase, which has been found to promote cell growth and to inhibit the apoptosis of tumor cells (Maceyka et al., 2004). ACY 1 deficiency was recently detected in lymphoblast cells from a young encephalopathic patient (Van Coster et al., 2005; Sass et al., 2006). Acylases also play an important role in the metabolism of the prodrug *N*-acetyl-L-cysteine (NAC), used in clinical practice to treat lung disorders (De Flora et al., 1995), and thus promote glutathione synthesis (Lauterburg et al., 1983).

ACY 1 has been reported to be involved in xenobiotic bioactivation as well as in the interorgan processing of *N*-acetyl-amino acid-conjugated xenobiotic derivatives (*S*-substituted-*N*-acetyl-L-cysteine) (Uttamsingh et al., 1998). The transport of acetylated amino acids found to occur across the rabbit intestinal epithelium has suggested the involvement of ACY 1 in protein metabolism (Brachet et al., 1991; Wallace et al., 1998). It was also recently suggested that ACY 1 may play a protective role by degrading bacterial and mitochondrial *N*-formylated peptides in the rat intestine and may constitute the first line of defense against constant exposure to commensal bacteria (Nguyen and Pei, 2005).

Certain features related to protein metabolism in the intestine are relatively unique to that tissue. Intestinal protein metabolism will be considered as occurring by two major mechanisms: (1) the formation, migration, differentiation and loss of whole cells, and (2) intracellular synthesis and degradation. In keeping with these two major mechanisms, the rat intestinal crypt–villus axis represents a useful model for study of the involvement of ACY 1 in *N*-acetylated protein/peptide metabolism, which represents a major event in the cell.

Therefore, in the present study, ACY 1 enzyme activity was investigated at the cellular level in the rat small intestine along the enterocyte–differentiation axis. We showed that ACY 1 is expressed at a high level in the undifferentiated crypt cells, from the absorptive lineage. In addition, we showed that ACY 1 expression in the rat intestinal crypt–villus axis is controlled at the mRNA level. Understanding ACY 1 cellular localization and expression in the intestine could give us indications on function(s) associated with this enzyme in this tissue.

2. Materials and methods

2.1. Materials

N-Acetyl-L-methionine, *p*-nitrophenyl phosphate, L-leucine-*p*-nitroalanine, L-amino acid oxidase, horseradish peroxidase (type II), *o*-dianisidine were obtained from Sigma (St. Louis, MO, USA).

2.2. Preparation of intestinal epithelial cells

Adult male Wistar rats weighing 260–300 g were used in this study. A 30-cm segment of small intestine was removed, and villus and crypt epithelial cells were prepared directly by shaking the intestinal segment for successive periods in a solution of EDTA as previously described in detail (Weiser, 1973). Briefly, after removal, the intestine segment was rinsed thoroughly with ice-cold phosphate-buffered saline (PBS), and flushed twice with ice-cold PBS containing 1 mM DTT. The segment was incubated at 37 °C for 15 min in a citrate buffer, pH 7.3, containing 96 mM NaCl, 1.5 mM KCl, 27 mM Na citrate, 8 mM KH_2PO_4 and 5.6 mM Na_2HPO_4 (Buffer A), then everted, filled and incubated at 37 °C in Buffer B (PBS buffer containing 1.5 mM EDTA, 0.5 mM DTT). The intestinal segment was gently shaken at 37 °C to remove adhering enterocytes from the villus–crypt axis, for nine incubation steps (F1–F9) after 4, 2, 2, 3, 4, 5, 7, 10 and 15 min, respectively. The cells from each fraction were collected by pelleting, washed twice in 10 ml fresh ice-cold PBS.

2.3. Enzyme activities

Immediately after isolating the cells, the enzyme activities were measured. The cells were lysed with an Ultraturax for 10 s in ice-cold PBS buffer, the resulting homogenate was centrifuged at 100,000 × *g* for 30 min and the supernatant was directly used for enzyme activities determinations.