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Comparative Biochemistry and Physiology, Part B 146 (2007) 124–130
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www.elsevier.com/locate/cbpb

Disaccharidase activities in camel small intestine: Biochemical investigations of maltase–glucoamylase activity

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Received 7 June 2006; received in revised form 4 October 2006; accepted 5 October 2006
Available online 6 October 2006

Abstract

Disaccharidases (maltase, cellobiase, lactase, and sucrase), α-amylase, and glucoamylase in the camel small intestine were investigated to integrate the enzymatic digestion profile in camel. High activities were detected for maltase and glucoamylase, followed by moderate levels of sucrase and α-amylase. Very low activity levels were detected for lactase and cellobiase. Camel intestinal maltase–glucoamylase (MG) was purified by DEAE-Sepharose and Sephacryl S-200 columns. The molecular weight of camel small intestinal MG4 and MG6 were estimated to be 140,000 and 180,000 using Sephacryl S-200. These values were confirmed by SDS-PAGE, where the two enzymes migrated as single subunits. This study encompassed characterization of MGs from camel intestine. The Km values of MG4 and MG6 were estimated to be 13.3 mM and 20 mM maltose, respectively. Substrate specificity for MG4 and MG6 indicated that the two enzymes are maltase–glucoamylases because they catalysed the hydrolysis of maltose and starch with α-1,4 and α-1,6 glycosidic bonds, but not sucrose with α-1,2 glycosidic bond which was hydrolyzed by sucrase–isomaltase. Camel intestinal MG4 and MG6 had the same optimum pH at 7.0 and temperature optimum at 50 °C and 40 °C, respectively. The two enzymes were stable up to 50 °C and 40 °C, followed by strong decrease in activity at 60 °C and 50 °C, respectively. The effect of divalent cations on the activity of camel intestinal MG4 and MG6 was studied. All the examined divalent cations Ca^{2+}, Mn^{2+}, Mg^{2+}, Co^{2+} and Fe^{3+} had slight effects on the two enzymes except Hg^{2+} which had a strong inhibitory effect. The effect of different inhibitors on MG4 and MG6 indicated that the two enzymes had a cysteine residue.

Keywords: Camel; Small Intestine; Disaccharidases; Maltase–Glucoamylase; Purification; Characterization

1. Introduction

Camels are very versatile animals. Camel are well adapted to life in the desert because of their unique metabolic pathways which enable the animals to survive without food and water for a few days (Haghkhah and Madjlesi, 1999). Their capacity to thrive well and reproduce in semi-arid and arid areas, where other livestock hardly survive, makes them the most important domestic animal in these areas (Woldemeskel et al., 2001). Around the world, camels are kept for breeding, various agricultural operations, milk and meat production and transportation (Azwai et al., 1996). In the Middle East, camels are also reared as racing animals. Breeding and management of racing camels in itself is a very lucrative business (Tinson et al., 2000). Very little information of digestion of camel especially for degradation of disaccharides in small intestine has been reported (Sir El Khatim and Osman, 1982).

The intestinal disaccharidases are enzymes present in the brush-border membrane of small intestinal epithelial cells. In several mammals, it has been shown that changes in disaccharidases activities occurred in order to optimize energy food intake (Sabat et al., 1995; Fan et al., 2002; Debray et al., 2003; Sabat and Veloso, 2003). The specific activities of these enzymes exhibited a heterogenous distribution along the intestine (del Valle et al., 2004). These enzymes participate in the degradation of dietary carbohydrates (Dahlqvist, 1974; Dahlqvist and Semenza, 1985). The structural features of sucrase–isomaltase, lactase–phlorizinase (LP), and trehalase have been deduced from genomic sequence analysis (Ruf et al., 1990; Chandrasena et al., 1994) as well as biochemical analysis of the proteins isolated from several animal species (Hunziker et al., 1986; Hu et al., 1987). Those studies have revealed a considerable structural homogeneity among the

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1096-4959/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpb.2006.10.001

corresponding enzymes in most mammal species in which they have been analyzed.

However, the structural features of maltase–glucoamylase (MG) appear to vary significantly among species. For example, biochemical and genomic analyses of MG from humans, pigs, and chickens have shown that the enzymes consist of a single polypeptide chain with a molecular mass of 335, 240 and 240 kDa, respectively (Noren et al., 1986; Nichols et al., 1998). In contrast, MG derived from rats and rabbits has been shown to be formed by a large multi-subunit protein with an apparent molecular mass of 530 and 700 kDa, respectively (Flanagan and Forstner, 1978; Lee et al., 1980). In addition, Quezada-Calvillo et al. (1993) reported that murine MG displays an apparent molecular mass of more than 500 kDa, suggesting a structure related to that reported for rats and rabbits.

Enzyme substrate specificities of isomaltase overlap with those of MG. *In vivo*, isomaltase accounts for 80% of maltase (1,4-*O*-α-D-glucanohydrolase) activity, all sucrase (D-glucopyranosyl-*β*-D-fructohydrolase) activity, and almost all isomaltase (1,6-*O*-α-D-glucanohydrolase) activity (Semenza and Auricchio, 1989). MG accounts for all glucoamylase exoenzyme (1,4-*O*-α-D-glucanohydrolase) activity for amylose and amylopectin substrates, 1% of isomaltase activity, and 20% of maltase activity (Semenza and Auricchio, 1989). Some have hypothesized that human mucosal glucoamylase exoenzyme activity is an alternate pathway for starch digestion when luminal α-amylase endoenzyme activity is reduced because of immaturity and malnutrition and that MG plays a unique role in the digestion of malted dietary oligosaccharides used in food and beverage manufacturing (Rossiter et al., 1974).

In the previous work, we reported that the disaccharidases (sucrase, maltase, cellobiase, lactase, trehalase), glucoamylase and α-amylase are existing in camel pancreas (Mohamed et al., 2005). The present study analyzed the existence of these enzymes in camel small intestine to integrate the enzymatic digestion profile in camel. In addition, the comparison of the chemical and physical characterization of two isoenzymes of maltase–glucoamylase was studied in order to understand their physiological role in the process of digestion in camel intestine.

2. Materials and methods

2.1. Experimental animals

Small intestines were collected of six healthy camels (*Camelus dromedaries*) from slaughter house, supervised by veterinary physicians.

2.2. Extraction of camel small intestine enzymes

Thawed samples from small intestine were cut in small pieces, weighed, thoroughly washed by cold physiological saline solution (NaCl 0.9 g/L) and suspended (small intestine 1 g contained 10 mg protein /10 mL) in 50 mM Tris-HCl buffer, pH 7.0. The suspension was homogenized for 3–5 min in an Omni Mixer, centrifuged for 10 min at 10,000 ×*g* and the supernatant collected after filtration through cheesecloth.

2.3. Disaccharidase assays

Maltase, cellobiase, lactase and sucrase activities were determined by measurement of released glucose. The reaction mixture in 1.0 mL contains: 50 mM sodium acetate buffer, pH 5.5, 28 mM substrate (maltose, CM-cellulose, lactose and sucrose) for the above listed enzymes, respectively, appropriate amount of the prepared crude extract (Sir El Khatem and Osman, 1982). Assays were incubated at 37 °C for 1 h. The reaction was stopped by heating in a boiling water bath for 10 min. The amount of glucose was determined using Glucose Enzymatic Kit of BioMerieux. One unit of disaccharidase activity was defined as 1 μmol glucose released per hour under standard assay conditions. Modified DNS reagent containing Rochelle salt (potassium sodium tartrate) was used for measurement of reducing sugar according to Fischer and Kohtes (1951).

2.4. α-amylase and glucoamylase assays

α-amylase and glucoamylase activities were determined by measurement of maltose and glucose, respectively, released from amylose according to Bernfeld (1951). The incubation was made at 39 °C for 10 min in tubes containing 12 mg potato soluble starch, 50 mM sodium acetate buffer, pH 5.5, appropriate amount of enzyme preparation and distilled water to give a final volume of 1.0 mL. The reaction was stopped for α-amylase by the addition of 0.5 mL DNS reagent, followed by incubation in a boiling water bath for 10 min then cooling and addition of 0.33 mL 40% sodium tartarate. The absorbance was recorded at 560 nm. For glucoamylase the reaction was stopped by heating the reaction mixture in a boiling water bath for 10 min and assayed for glucose released by Enzymatic Glucose Kit of BioMerieux. One unit of α-amylase or glucoamylase was defined as 1.0 μmol maltose or glucose released per hour under standard assay conditions, respectively.

2.5. Purification of camel intestinal maltase-glucoamylase

Unless otherwise stated all steps were performed at 4–7 °C using 50 mM Tris-HCl buffer, pH 7.0. Crude extract was prepared as mentioned above in the extraction of small intestinal enzymes. The crude extract was then applied directly to a DEAE-Sepharose column (10 × 1.6 cm i.d.) equilibrated with 50 mM Tris-HCl buffer, pH 7.0. The exchanged material was eluted with a stepwise gradient ranging from 0.0 to 0.4 M NaCl prepared in the same buffer at a flow rate of 60 mL/h and 3 mL fractions. Protein fractions exhibiting maltase–glucoamylase activity were pooled in six peaks (MG1–MG6). MG4 and MG6 DEAE-Sepharose fractions were applied separately to a Sephacryl S-200 column (90 × 1.6 cm i.d.) equilibrated with the same buffer and developed at a flow rate of 20 mL/h and 2 mL fractions. The maltase–glucoamylase was eluted with the same buffer.

2.6. Protein determination

Protein was determined either by measuring the absorbance at 280 nm (Warburg and Christian, 1942) or by the method of Bradford (1976) using bovine serum albumin as a standard.