Expression of DNase I in Rat Parotid Gland and Small Intestine Is Regulated by Starvation and Refeeding

(Manuscript received 31 August 2002. Initial review completed 29 September 2002. Revision accepted October 9, 2002.)

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ABSTRACT DNase I in rats is mainly expressed in the parotid gland and the small intestine and functions as a digestive enzyme. Male Wistar rats were deprived of food for 48 h, refed with nonpurified diet for 2 h and killed at 0, 0.33, 0.67, 1, 2, 6 or 12 h. The activity and mRNA of DNase I in the parotid gland and the small intestine were determined. We found that in rats that were not fed for 48 h there was accumulation of DNase I in the parotid gland but not in the small intestine. In the parotid gland, refeeding decreased DNase I activity (< 0.05), perhaps due to an increase in secretion. The increase in DNase I mRNA probably resulted from the need for protein synthesis. However, in the small intestine, both the enzyme activity and the amount of mRNA were up-regulated by refeeding (< 0.05). Exposing rats to food in a sealed transparent flask also caused a 2.5-fold increase in DNase I mRNA within 30 min in the parotid gland. These data suggested that the expression of rat parotid DNase I is up-regulated by feeding and that mastication is not essential for the regulation. J. Nutr. 133: 71–74, 2003.

KEY WORDS: • DNase I • parotid gland • small intestine • starvation • refeeding

DNase I (EC 3.1.21.1.) requires Mg2+ or Mn2+ for its catalytic activity, has an optimal pH of ~7–8 and produces 5'-phosphate nucleotides on hydrolysis of DNA. DNase I has been found mainly in the pancreas of most species of vertebrates studied (1). Bovine pancreatic DNase I is the most thoroughly studied DNase I and consists of a single polypeptide with a molecular weight of 31,000 (2). However, in rats DNase I is found mainly in the secretory cells of the parotid gland and not in the pancreas. The small intestine of rats also expresses a substantial amount of DNase I in the enterocytes covering the villi (3,4). The cDNA sequences of human, rat and mouse DNases I have been cloned and sequenced (5–7).

These available cDNA sequences facilitated our studies on the regulation of DNase I at the gene expression level under various physiological conditions.

DNase I has been implicated in programmed cell death (apoptosis) (8) and has been studied intensively in this field. However, DNase I as a digestive enzyme that is physiologically regulated is poorly understood. Although nucleotides are not essential nutrients as are some amino acids, several studies have suggested that dietary nucleotides appear to be important in supporting cellular metabolism and function, particularly in rapidly dividing tissues such as lymph and intestine (9–11). Nucleotides are present in regular diets, and an adult consumes 1–2 g of nucleotides/d in normal food (12) in the form of DNA or RNA. Nucleases are required to degrade DNA and RNA before they can be absorbed by the body. DNase I is mainly responsible for the digestion of dietary DNA. In this study we investigated the regulation of the DNase I activities and the levels of their mRNA expression in the parotid gland and the small intestine during starvation and refeeding in rats.

MATERIALS AND METHODS

Animals and treatment. Male Wistar rats weighing 160–170 g, obtained from the Animal Center of the National Taiwan University (Taipei, Taiwan), were housed in groups of three or four in stainless steel cages on a 12-h light cycle (0700–1900 h) with free access to a nonpurified diet (Purina Certified Rodent Chow 5001; Ralston-Purina, St. Louis, MO) and tap water for 10 d before the experiments. Rats were not fed for 48 h, were fed nonpurified diet at 0900 h for 2 h and were killed in a CO2 chamber at 0, 0.33, 0.67, 1, 2, 6 or 12 h after refeeding. Experimental groups consisted of three rats except there were four rats in the control group. In a separate experiment, nonpurified diet in a sealed transparent flask was placed in the cage of the starved rats, with the diet visible but not available for eating. These rats were killed 30 and 60 min later. Water was given ad libitum during all the experiments. Immediately after the rats were killed, parotid glands and a 2-cm segment of the small intestine from each rat were removed from the part just distal to the stomach. The tissues were frozen immediately in liquid nitrogen and stored at −70°C until measurements for DNase I activity and the amount of mRNA. All animal experimental procedures followed the "Guide for the Care and Use of Laboratory Animals" of the National Science Council, Taiwan.

DNase I assay. Parotid gland or small intestine tissue was homogenized in cold buffer containing 0.1 mol of Tris-HCl (pH 7.0) and 20 mmol of CaCl2 per L and centrifuged for 20 min at 13,000 × g; the supernatant was kept for activity assay. DNase I activity in the parotid gland was determined by the hyperchromicity assay as described (13). DNase I activity in the small intestine was relatively low and was determined by a fluorescence assay. This high sensitivity assay method was carried out as follows. Crude extract of the small intestine containing 300 µg of protein was added to each well of a 96-well Corning plate that contained 0.25 µmol of ethidium bromide, and 2 g of DNA in reaction buffer [100 mol of Tris-HCl (pH 7.2), 10 mol of CaCl2, 10 mol of MnCl2, and 500 mg of bovine serum albumin per L] per L in a total volume of 200 µL. Fluorescence was measured every 150 s with excitation at 485 nm and emission at 645 nm on a CytoFluor 2300 Fluorimeter (Millipore, Bedford, MA). The fluorescence intensities were plotted against time. These kinetic...
DNase I activity in the parotid gland and small intestine of rats deprived of food for 48 h and refeed

<table>
<thead>
<tr>
<th>Time after refeeding, h</th>
<th>0.33</th>
<th>0.67</th>
<th>1</th>
<th>2</th>
<th>6</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed control</td>
<td>13.6 ± 1.8</td>
<td>29.3 ± 2.2</td>
<td>19.4 ± 2.3</td>
<td>11.8 ± 3.0</td>
<td>9.6 ± 2.9</td>
<td>10.6 ± 1.7</td>
</tr>
<tr>
<td>Starved for 48 h</td>
<td>1.8 ± 0.2</td>
<td>2.0 ± 0.5</td>
<td>2.5 ± 0.2</td>
<td>3.6 ± 0.7</td>
<td>3.8 ± 0.6</td>
<td>3.8 ± 0.4</td>
</tr>
</tbody>
</table>

1 Values are given as mean ± SD, n = 3 or 4 (fed and starved controls). a Different from the 48-h starved control (P < 0.05). b Different from the fed control (P < 0.05). 2 ND, nondetected.

Effects of starvation and refeeding on the levels of DNase I mRNA in the parotid and the small intestine. Northern blot analysis detected a single band of DNase I mRNA, with the highest amount in the parotid gland and a smaller amount in the small intestine, and a barely detectable signal in the submaxillary gland and kidney (data not shown). The mRNA was not detected in the liver, stomach, pancreas, lung, heart, skeletal muscle, spleen, adipose, large intestine, lymph nodes, thymus or brain by Northern blot analysis. However, it was detected in the spleen, liver, lymph nodes and thymus by reverse transcription polymerase chain reaction (data not shown).

The effects of starvation and refeeding on the levels of DNase I mRNA were determined in pools from three rats by slot-blot analysis. Although starvation led to the accumulation of DNase I activity in the parotid gland, only a small amount of DNase I mRNA could be detected during this time (Fig. 1). In response to feeding, DNase I mRNA increased very quickly and reached a peak of 12-fold that of starved control rats at 40–60 min. The mRNA decreased 25% from the peak in the next 1 h and then remained near a constant amount of ~80% of the peak value during the next 10 h. Thus in the parotid gland, the changes in DNase I mRNA occurred in reverse of those in the DNase I activity.

Similar to the parotid gland, DNase I mRNA was low in the small intestine after 48 h of starvation. The amount of DNase I mRNA was induced by feeding (Fig. 2). Although the response was not as fast as that in the parotid gland, intestinal DNase I mRNA reached a peak value of 9.9-fold that of starved controls at 1 h after refeeding. The amount of mRNA declined very quickly and returned to the basal level 10 h later.

DISCUSSION

DNase I is generally regarded as a digestive enzyme and is believed to be synthesized by the exocrine glands such as the pancreas or the parotid gland and secreted into the alimentary tract, where it carries out its digestive function. However, the functional role of DNase I in the small intestine and other nonsecretory tissues is not fully understood. Polzar et al. (4)

Abbreviation used: GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
have suggested that expression of DNase I in the small intestine and kidney is involved in cellular turnover and programmed cell death for these tissues. It is not clear whether DNase I is secreted into the alimentary tract from the small intestine. However, our data showed that intestinal DNase I activity and mRNA were clearly up-regulated by feeding. These data suggested that DNase I in the small intestine not only was involved in the apoptotic pathway but also might play an important role as a digestive enzyme.

DNase I in the parotid gland at a certain time point represents the balance of the secretion and synthesis of the enzyme. Therefore, a decrease in the enzyme activity in the tissue does not mean that synthesis of the enzyme is also decreased. There probably is a storage pool of DNase I in the parotid gland. The enzyme in this pool is secreted with saliva, resulting in a decrease in enzyme in the gland while eating. However, at the same time the amount of mRNA increases in the gland, suggesting that the synthesis of the enzyme is induced by feeding. It is possible that the secretion rate was higher than the synthesis rate for the enzyme, resulting in the decrease of DNase I in the parotid gland. In the small intestine, there probably is no storage pool of DNase I; the enzyme and mRNA of DNase I were low after 48 h of food deprivation, and the synthesis of the mRNA and protein was stimulated in response to feeding.

Sreebny and Johnson (18) have demonstrated that mastication plays an important role in regulating the synthesis of the secretary products of the parotid gland. In this study, we found that DNase I mRNA was up-regulated in rat parotid by feeding, and mastication might not be essential for the regulation because the amount of mRNA was increased 2.5-fold.
within 30 min when the diet was not offered but was seen in a sealed transparent flask (Fig. 3). It has been demonstrated that the synthesis and secretion of amylase in rat parotid gland are accelerated by parasympathetic and sympathetic nerve stimulation in vivo (19). It is possible that the synthesis and secretion of DNase I in parotid gland were also regulated, at least in part, by the nerve impulses.

LITERATURE CITED