BBA 61207

The cellular localization of enterokinase

Enterokinase (enteropeptidase, EC 3.4.4.8) initiates the pancreatic digestion of dietary protein by proteolytic conversion of trypsinogen into trypsin, which in turn activates the other pancreatic proenzymes, chymotrypsinogens and procarboxypeptidases. Thus enterokinase is a key enzyme for the utilization of dietary protein, the absence of which implies severe clinical symptoms[1].

It is a general concept that enterokinase is formed in the small intestinal mucosa and therefrom secreted into the lumen of the intestine[2]. Consequently, this enzyme has been studied in the duodenal juice as an indicator of the state of the intestinal mucosa in gastrointestinal diseases and after pharmaceutical and radiation therapy. The mechanism of formation of trypsin from trypsinogen by means of enterokinase has also been studied *in vitro* by different investigators starting with the pioneer work of KUNITZ[3].

The literature, however, lacks information on the site of enterokinase formation in the intestinal wall. The enzyme may be formed in the villi, in the crypts or possibly in the Brunner glands. We have therefore quantitatively studied the localization of enterokinase within the duodenal wall of the rat by a cryostat cutting technique which has previously been used for similar studies with other enzymes[4–6]. This method allows the isolation of different parts of the villi and crypts by horizontal sectioning of fresh frozen pieces of the intestinal wall.

Enterokinase activity was measured by a micromodification of the method of HADORN *et al.*[1], which in turn is based on the original method of KUNITZ[3]. Crystalline bovine trypsinogen was used as the substrate and the increase in trypsin activity was measured by the chromogenic method of ERLANGER *et al.*[7]. A unit of trypsin is the activity hydrolyzing 1 μmole of benzoyl DL-arginine *p*-nitroanilide·HCl per min. A unit of enterokinase is the activity activating 1 unit of trypsin per min. Incubations were performed at 25°.

Adult albino rats were narcotized with ether and the duodenum was cut out and rinsed with saline (1.3 ml/cm of intestine), and then pieces from different levels were taken for cryostat sectioning. Most of the trypsin activity (about 90%) but only a small fraction of the enterokinase activity (about 5%) was removed by the saline rinsing. The residual trypsin activity in the intestinal wall, after appropriate dilution of the sample, was too small to disturb the determination of enterokinase. When the low enterokinase activity of the perfusate was to be assayed, however, the presence of high activity of trypsin influenced to some extent by autocatalysis (trypsin activating trypsinogen). This was not corrected for, and the figure 5% for enterokinase activity in the perfusate will therefore even be somewhat too high and should be regarded as a maximum figure.

The localization of enterokinase in the different parts of the villi and crypts is seen in Fig. 1. The highest specific activities were found in the apical halves of the villi, and the activity decreased steeply towards the crypts. Within the crypts there was no or negligible activity (possibly very weak activity at the mouths of the crypts). No activity was found in the Brunner glands of the duodenum.

The distribution profile for enterokinase is thus of the same principal type as

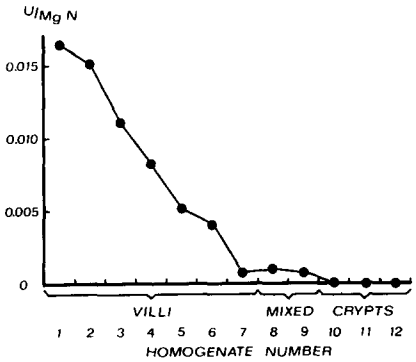

Fig. 1. Distribution of enterokinase activity (units/mg nitrogen) within the wall of rat duodenum. Each homogenate contains six 20-μ-thick sections, serially cut from the tips of the villi to the serosa. The last sections also contain submucosal tissue.

has previously been found for several digestive enzymes[4–6], which on the subcellular level are located in the brush borders of the villous epithelial cells.

The results show that enterokinase is a villous enzyme, at least in the rat, and it therefore seems probable that the function of the enzyme, as for the other villous enzymes, is confined to the epithelial cells. Trypsinogen would then be activated by enterokinase on the surface membrane of the cells rather than in the lumen as has hitherto been thought. The enterokinase found in the juice then seems to be released by cell desquamation at the tips of the villi, rather than by secretion. Subcellular fractionation studies in progress in our laboratory have also supported the idea that enterokinase is a brush border enzyme.

This investigation has been supported by grants from the Medical Faculty of the University of Lund, the Swedish Nutrition Foundation, Albert Påhlsson Foundation and the Swedish Medical Research Counsil (Project No. B70-13P-2262 and B70-13X-157). The authors are indebted to Miss Ulla Iwarson for her skillful technical assistance.

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Received December 22nd, 1969