DISTRIBUTION OF THE SALIVARY PROTEOLYTIC ENZYME SALIVAIN IN THE DIGESTIVE SYSTEM OF RATS

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A method is suggested for determining the activity of salivain, a trypsin-like enzyme of the saliva, by using its ability to split benzoyl-arginine-p-nitroanilide in the presence of soy trypsin inhibitor. Of all the organs in the digestive system of rats which were studied, the submandibular gland has the highest salivain activity. Salivain is found in the contents of the stomach, the small intestine, and the cecum, and also in the feces. Salivain is resistant to the action of strongly acid media, trypsin, chymotrypsin, pepsin, and enteropeptidase.

The presence of proteolytic enzymes in the saliva was established some time ago [3, 10], but it is only recently that they have been subjected to detailed biochemical study [6]. Salivain, a trypsin-like enzyme of the saliva, has been described in detail [8, 9]. The substrate specificity of salivain is unusually similar to that of trypsin, but the specific activity of salivain as regards the hydrolysis of synthetic substrates is 10 times higher than that of trypsin.

Considering that according to some investigators [7] the proteolytic enzymes of the saliva digest up to 50% of the food proteins in rodents, and assuming that the action of salivain takes place not only or, indeed, not chiefly in the mouth it was decided to investigate the distribution of salivain in the digestive system of rats.

EXPERIMENTAL METHOD

Female Wistar albino rats weighing 180-245 g were used in the experiments. Saliva was collected under pentobarbital anesthesia (20 mg/kg) in response to stimulation by pilocarpine (5 mg/kg) by the method of Benarde et al. [4]. The contents of the stomach, small intestine, and cecum were obtained by washing out the corresponding portions of the digestive tract with cold 0.85% NaCl solution immediately after the animals were killed by total exsanguination from the heart. The contents were carefully homogenized, their total volume measured, and centrifuged at 1000 g (15 min at 0°C). To determine salivain activity the supernatant was used. The pancreas, the parotid, submandibular, and large sublingual glands, the glandular part of the stomach, and the proximal portion of the jejunum also were taken from the rats. The tissues were homogenized in the proportion of 20 mg wet weight to 1 ml.

TABLE 1. Activity of Salivain (in μmoles p-nitroaniline/g dry weight/min) in Digestive Organs of Albino Rats (M ± m)

Test object	Activity (n = 12)
Parotid gland	0,011±0,001
Submandibular	7,61±0,304
Sublingual	0,070±0,003
Stomach	0,043±0,008
Liver	0,032±0,016
Pancreas	0,227±0,054
Jejunum	0,067±0,008

Note: Here and in Table 2, n denotes number of experiments.

To determine the salivain activity the specific substrate benzoyl-arginine-p-nitroanilide (BAPN) was used; this compound is split by trypsin and salivain but not by chymotrypsin, pepsin, or enteropeptidase, and it is also split in the presence of soy trypsin inhibitor, which does not affect the activity of

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TABLE 2. Activity of Salivain (in μmoles p-nitroaniline/min) in Contents of Various Parts of the Digestive Tract of Albino Rats (M ± m)

Test object	Activity (n = 8)	
	per gram dry wt.	for the whole portion
Saliva	1,52±0,13	—
Contents of stomach	0,015±0,004	0,0055±0,0026
Contents of small intestine		
proximal third	0,077±0,012	0,0003±0,0001
middle third	0,086±0,013	0,0013±0,0003
distal third	0,150±0,029	0,0017±0,0007
Contents of cecum	0,066±0,013	0,0010±0,0003
Feces	0,030±0,008	—

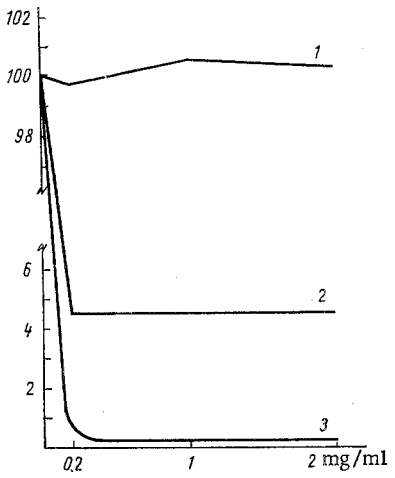

Fig. 1. Effect of soy trypsin inhibitor on BAPN-amidase activity of saliva (1), contents of small intestine (2), and crystalline trypsin (3). Abscissa, concentration of soy trypsin inhibitor (in mg/ml); ordinate, BAPN-amidase activity (activity in absence of soy inhibitor taken as 100%).

salivain. BAPN was synthesized from L-arginine, benzoyl chloride and p-nitroaniline [5]. Next, 21.8 mg BAPN was dissolved in 1 ml dimethylformamide, and the volume was made up to 100 ml with water. Before the determination, the BAPN solution was mixed, (1:1) with glycine buffer (0.1 M, pH 9.24). The incubation mixture consisted of 2.5 ml buffered solution of substrate, 0.5 ml enzyme diluted 1:2 with soy trypsin inhibitor (Reanal, Hungary) solution (1 mg/ml), and the extinction was then immediately measured at 382.5 nm. After incubation for 60 min at 25°C the extinction of the mixture was again measured. Activity of the enzyme was expressed in μmoles p-nitroaniline formed in 1 min by 1 g dry weight. To study the effect of proteases on salivain activity, pepsin (Schuchardt, West Germany), trypsin (Spofa, Czechoslovakia), and α-chymotrypsin (USSR) were used, and an extract from 50 mg acetone powder of hog intestine was used as enteropeptidase. The solutions of these proteases (pepsin in 0.1 M glycine-HCl buffer, pH 2.0; trypsin in 0.05 M Tris-HCl buffer, pH 7.5; α-chymotrypsin in the same buffer and enteropeptidase in 0.05 M Tris-HCl buffer, pH 7.2) were incubated for 1 h at 37°C with an equal volume of rat saliva (the source of salivain). After preincubation in this way the salivain activity was determined as described above.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the soy inhibitor did not affect the BAPN-amidase activity of the saliva (salivain), reduced the activity of the contents of the small intestine (trypsin + salivain) by 95.5%, and inactivated trypsin virtually completely (by 99.8%). Results of the determination of salivain activity in the digestive organs of albino rats are given in Table 1. Clearly the submandibular glands possessed the highest salivain activity. It follows from Table 2 that, as might be expected, the salivain activity was highest in the saliva. The maximal activity in the small intestine was concentrated in the distal third, in agreement with the authors' findings for trypsin and chymotrypsin [2]. If calculated per unit weight of the part concerned, the greatest salivain activity was found in the stomach. A certain quantity of salivain was excreted with the feces. This fact indicates that salivain is resistant to the environmental conditions of the stomach and intestine. To test this hypothesis the effect of preincubation of salivain from rat saliva with various acid solutions and proteolytic enzymes was studied. The tests showed that salivain activity was virtually unchanged in strongly acid media (pH 2.0-7.54), and in the presence of pepsin (1 mg/ml), enteropeptidase, trypsin (0.05 mg/ml), and chymotrypsin (0.1 mg/ml).

Salivain is thus a highly stable proteolytic enzyme capable of performing its digestive functions not only in the mouth, but also in the lower portions of the gastro-intestinal tract.

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