DETERMINATION OF SERUM PEPSINOGENS WITH SYNTHETIC SUBSTRATES

L.KORBOVÁ[a], J.KOHOUT[b], E.KASAFÍREK[c] and J.ČÍŽKOVÁ[a]

[a] *Institute of Pathological Physiology,*
[b] *1st Department of Surgery, Faculty of General Medicine,*
Charles University, 120 00 Prague 2 and
[c] *Research Institute for Pharmacy and Biochemistry, 130 60, Prague 3*

Received June 6th, 1976

The presence of zymogens of the individual gastric proteinases has been demonstrated in human serum. Earlier experience with the determination of acid gastric proteinases of the pepsin and gastricsin type (group I and II proteinases) served as a basis of the development of a procedure for determination of both pepsinogen groups in serum.

Pepsinogens identical to those present in gastric mucosa can be detected in serum[1,2]. Samloff[1,2] developed a method of determination of pepsinogens of group I which according to common nomenclature[3] are precursors of pepsin. These are pepsinogens originating in the stomach which give rise to proteinases with pH-optimum of activity around 1·8. Pepsinogens of group II (precursors of gastricsin) have so far been determined in serum and urine by electrophoretic and chromatographic techniques[1,4]. Pepsinogens of group II — unlike those of group I — can be detected in human urine of 2% of normal individuals[4,5]. The relation between the level of total pepsinogens in the serum and gastric secretion is being studied[6,7].

In the preceding study[8] we have shown that gastric proteinases of group II, which have the character of gastricsin, cleave synthetic substrates formyl-L-tyrosyl-L-tyrosine and formyl-L-tyrosyl-L-phenylalanine. We have tried to develop a method of direct determination of pepsinogens of group I (proenzymes of pepsin), based on the use of acetyl-L-phenylalanyl-L-diiodotyrosine as substrate, and of pepsinogens of group II (proenzymes of gastricsin) with formyl-L-tyrosyl-L-tyrosine and formyl-L-tyrosyl-L-phenylalanine as substrate.

EXPERIMENTAL

Material. The blood was collected from fasting healthy individuals, from patients with a peptic duodenal ulcer, from patients who showed no secretion of free hydrochloric acid even after the administration of histamine, and from patients after total gastrectomy. In the second series of experiments serum pepsinogens were examined simultaneously with gastric proteinases in gastric juice after secretion stimulation with pentagastrin (Gastrodiagnost Merck). In the third

series of experiments serum pepsinogens were examined in healthy fasting individuals, and 20, 40, and 60 min after food intake.

The activation of serum pepsinogens was effected by the addition of 0·4M-HCl at a ratio of 1 : 1 to the serum assayed; the mixture was allowed to stand 30 min at room temperature. The adjusted serum (0·5 ml) and 0·15 ml of 2 mM substrate solution in acetate buffer at pH 5·3 were incubated for 24 h at 37°C. When the serum proteins are precipitated by trichloroacetic acid from the serum the substrates are simultaneously hydrolyzed. We used therefore sodium trichloroacetate at pH 3·5 for the precipitation of proteins. Synthetic substrates with a formyl group are hydrolyzed at low pH and prolonged incubation (Table I). We used such a ratio of serum, 0·4M hydrochloric acid, and substrate solution at which the final pH was about 3·0, *i.e.* where hydrolysis does not proceed. This pH-value lies in the optimum range of cleavage of the substrates used.

Determination of proteinase activity. The samples were centrifuged (20 min, 3 600*g*) and 0·25 ml of the supernatant was treated with 0·5 ml of 4M acetate buffer at pH 5·5 and 0·5 ml of the ninhydrin reagent. The amino acids liberated were determined by the method of Rosen[9].

RESULTS AND DISCUSSION

When acid gastric proenzymes are activated in serum and during their incubation with synthetic substrates, serum proteins are simultaneously digested. Serum with activated enzymes incubated for 24 h in the absence of substrate served as a blank solution (Fig. 1). To check whether pepsinogens of group I and II cleave specifically the substrate under these conditions we carried out experiments with activated serum to which gastricsin and pepsin had been added in three different concentrations (Fig. 2). Under these conditions proteinases of group I cleaved exclusively

TABLE I

Chemical Hydrolysis of Synthetic Substrates Incubated 24 h with Inactivated Human Serum

Following activation of serum pepsinogens by acidification of the serum with 0·4M-HCl (pH 2·0, 30 min), the acid proteinases were inactivated by adjustment of pH to 10·0; after a 60-min period, the serum was again acidified to pH 2·0 or 3·0, respectively. Differences in absorbance at 570 nm of inactivated sera at pH 2·0 and 3·0 are due to chemical hydrolysis of the substrates in acid medium.

Substrate	Hydrolysis of substrates, A_{570}		
	active serum	inactivated serum	
		pH 2·0	pH 3·0
Ac-L-Phe-L-Tyr(I_2)	0·535	0·205	0
For-L-Tyr-L-Tyr	0·610	0·220	0
For-L-Tyr-L-Phe	0·865	0·625	0·090

acetyl-L-phenylalanyl-L-diiodotyrosine and proteinases of group II formyl-L-tyrosyl-L-tyrosine and formyl-L-tyrosyl-L-phenylalanine.

In patients which showed no secretion of free hydrochloric acid after stimulation, both groups of pepsinogens were statistically decreased; pepsinogens of group I are practically not present in the serum at all. Only traces of pepsinogens of group II

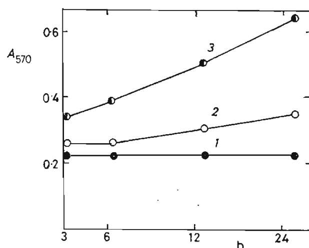

Fig. 1

Activity of Serum Pepsinogens Activated by Acidification of Serum to pH 2·0

1 Activity of serum ($A_{570\text{nm}}$), stored at 0°C, *2* activity of serum incubated at 37°C with activated pepsinogens (pH 2·0), and *3* activity of mixture of serum and formyl-L-tyrosyl-L-tyrosine at 37°C at identical time intervals (h).

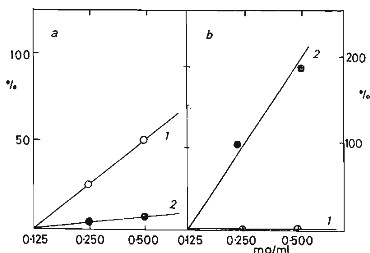

Fig. 2

Cleavage of Synthetic Substrates in Activated Serum after Addition of Pepsin (*a*) and Gastricsin (*b*)

1 Cleavage of acetyl-L-phenylalanyl-L-diiodotyrosine, *2* cleavage of formyl-L-tyrosyl-L-tyrosine. The values are given in per cent of activity of the original activated serum before the addition of pepsin or gastricsin, respectively (mg/ml).

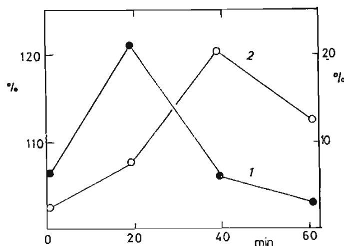

Fig. 3

Rise of Level of Pepsinogens of Group I and II after Food Intake

The changes in activity are expressed in per cent of starting values of A_{570} obtained from fasting patients. The examination was carried out with 10 individuals 20, 40, and 60 min after food intake. *1* Changes in activated pepsinogens of group I (cleavage of acetyl-L-phenylalanyl-L-diiodotyrosine) and *2* changes in activated pepsinogens of group II (cleavage of formyl-L-tyrosyl-L-tyrosine).

TABLE II

Comparison of Serum Pepsinogens with Gastric Proteinases Determined Simultaneously

Gastric secretion was stimulated by pentagastrin (6 μg per kg of body weight, intravenously). Gastric proteinases and serum pepsinogens were examined in fasting patients and at various intervals after the administration of pentagastrin (total number 9 patients). The activity of serum pepsinogens and gastric proteinases of group I is given in terms of cleavage of Ac-L-Phe-L-Tyr (I_2), the activity of serum pepsinogens and of gastric proteinases of group II in terms of cleavage of For-L-Tyr-L-Tyr.

Conditions	Cleavage of Ac-L-Phe-L-Tyr(I_2), A_{570}		Cleavage of for-L-Tyr-L-Tyr, A_{570}	
	serum	gastric juice	serum	gastric juice
Hungry	0·284	0·281	0·222	0·345
Pentagastrin 30 min	0·345	0·297	0·200	0·305
Pentagastrin 60 min	0·369	0·235	0·146	0·290

were detected after total gastrectomy. The administration of pentagastrin to patients with a duodenal ulcer brought about an increase of concentration of proteinases of group I in gastric juice and simultaneously of pepsinogens of group I in serum. By contrast, the concentration of acid proteinases of group II in gastric juice and of pepsinogens of group II in serum decreases. The changes in the concentration of acid proteinases in gastric juice and of the corresponding pepsinogens in the serum are parallel for the first 30 min (Table II).

The concentration of both groups of pepsinogens in serum increases after food intake. These changes are not simultaneous. The concentration of pepsinogens of group II reaches a maximum 20 min later than the concentration of pepsinogens of group I (Fig. 3). We found no differences when serum pepsinogens were determined by the method of Anson and Mirsky[10].

The synthetic substrates specifically cleaved by the individual groups of gastric proteinases can be used also for the determination of the concentration of the precursors of these proteinases, *i.e.* of pepsinogen of group I and II in the serum. When this method is compared with others it becomes obvious that it is simple and suitable for routine tests. The results suggest that the method can be used also in studies on physiological and pathophysiological problems of gastric secretion of acid proteinases.

REFERENCES

1. Samloff I. M.: Gastroenterol. *60*, 586 (1971).
2. Samloff I. M.: Gastroenterol. *60*, 713 (1971).
3. Richmond V., Tang J., Wolf S., Trucco R. C., Caputto R.: Biochim. Biophys. Acta *29*, 453 (1958).
4. Samloff I. M., Towness P. L.: Gastroenterol. *58*, 462 (1970).
5. Hirsch-Marie H., Conte M.: Rev. Franc. Etud. Clin. Biol. *14*, 977 (1969).
6. Samloff I. M., Secrist D. M., Passaro D.: Gastroenterol. *69*, 1196 (1975).
7. Lombards A. J. P. F., Peters H. J.: Clin. Chim. Acta *36*, 195 (1972).
8. Korbová L., Kohout J., Kasafírek E., Čížková J.: This Journal, in press.
9. Rosen H.: Arch. Biochem. Biophys. *67*, 10 (1957).
10. Anson M. L., Mirsky A. E.: J. Gen. Physiol. *16*, 59 (1932).

Translated by V. Kostka.