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Note

Fractionation of human gastric proteinases by immobilized metal chelate (Fe^{3+}) affinity chromatography

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Porcine pepsin, in common with some other phosphoproteins, binds to ferric ions immobilized on Iminodiacetate (IDA)–Agarose gel and can be eluted by increasing the pH or by introducing phosphate ions into the eluent. These observations were utilized to develop a purification procedure for porcine pepsin [1]. Human gastric pepsins do not bind completely on this support, some human pepsins and pepsinogens are adsorbed on immobilized iminodiacetate ferric chelate, but others are free in the solution. Human pepsins, as a consequence, can be fractionated by this method.

EXPERIMENTAL

Iminodiacetate Ultrogel AC-34 from Pharmacia (Uppsala, Sweden), Separon HEMA 1000-DEAE from Tessek (Prague, Czechoslovakia), acrylamide and haemoglobin from Serva (Heidelberg, F.R.G.), agar from Difco (Detroit, MI, U.S.A.) and acid phosphatase from Reanal (Budapest, Hungary) were used. The activity of proteinases was determined by milk-clotting according to McPhie [2]. Electrophoresis on agar gel was carried out either in 1.5% agar and 0.025 *M* sodium acetate buffer (pH 5.0) at a potential gradient of 20 V/cm [3] or in 1.8% agar in 0.07 *M* Tris–glycine–veronal–HCl buffer (pH 8.3) at a potential gradient of 30 V/cm for pepsinogens [4]. Polyacrylamide gel electrophoresis (PAGE) was carried out according to Frants et al. [5]. The detection of proteolytic activity on zymograms was effected by 0.6% haemoglobin at pH 2.0. Dephosphorylation with potato acid phosphatase was performed at pH 5.5 (37°C, 16 h) according to Martin et al. [6].

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Preparation of samples

Fresh human gastric juice obtained from patients of the 2nd Department of Internal Medicine (Prague, Czechoslovakia) was adjusted to pH 3.5 with 0.2 *M* sodium acetate and centrifuged at 1000 *g* for 30 min. The supernatant was adjusted to pH 5.0 with 0.1 *M* sodium hydroxide. Extract of human gastric mucosa was obtained from resected parts of stomachs of patients suffering from gastric carcinoma and ulcer diseases on the 1st Surgical Clinic (Prague, Czechoslovakia). The extract of mucosa was prepared as described previously [7]. The supernatant of this extract was applied to a column of Separon HEMA 1000-DEAE equilibrated with 0.05 *M* Tris–HCl buffer (pH 7.2). Elution was effected with a 0.1 *M* sodium acetate buffer (pH 5.7) containing 0.5 *M* sodium chloride. The pepsinogen-containing fraction was used for metal chelate chromatography.

Chromatography

IDA–Agarose was packed into a 9 cm×0.6 cm I.D. column and 50 m*M* ferric chloride was applied. The column was washed with distilled water, then equilibrated with the starting buffer: 0.1 *M* sodium acetate (pH 5.0) for pepsin and 0.1 *M* sodium acetate (pH 5.7) for pepsinogen. Samples (2 ml in starting buffer) were applied to the IDA–ferric column and washed with the starting buffer. Elution was effected with the starting buffer containing 20 m*M* sodium phosphate.

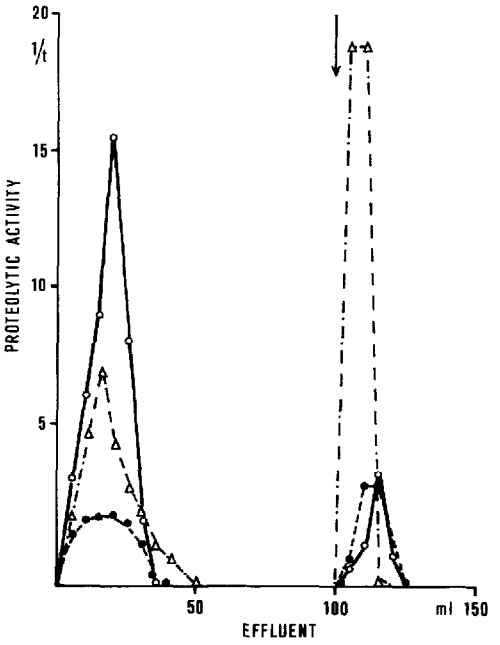

Fig. 1. Chromatography of human gastric juice of three patients on an IDA–ferric Ultrogel column. Samples in 0.1 *M* sodium acetate buffer (pH 5.0) were washed with the same buffer; the arrow indicates the starting buffer containing 20 m*M* sodium phosphate.

RESULTS AND DISCUSSION

Only some of human gastric pepsins (Fig. 1) and pepsinogens (Fig. 2) were sorbed on IDA–Agarose. According to agar gel electrophoresis and PAGE, pepsinogens and pepsins with higher electrophoretic mobilities were adsorbed. One peak only (Fig. 3) was observed after rechromatography of the unadsorbed portion. Pepsinogens, dephosphorylated with the use of acid phosphatase, are not adsorbed at all (Fig. 4). Proteolytically active proteins were adsorbed from hu-

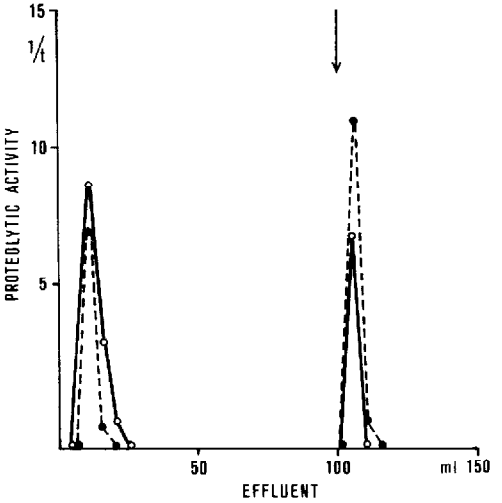

Fig. 2. Chromatography of the extract from human gastric mucosa of two patients on an IDA–ferric Ultrogel column. Samples in sodium acetate buffer (pH 5.7) were washed with the same buffer; the arrow indicates the starting buffer containing 20 m*M* sodium phosphate.

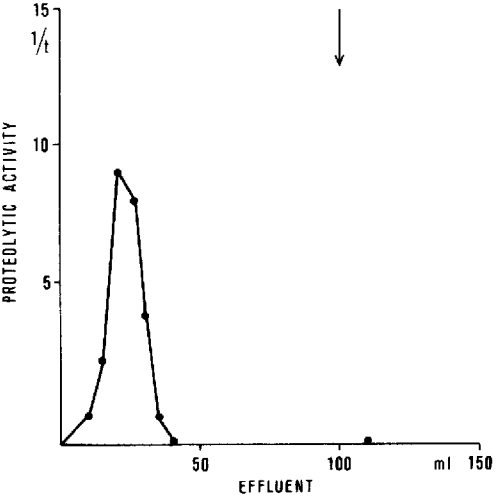

Fig. 3. Rechromatography of peak I from human gastric juice on an IDA–ferric Ultrogel column. Samples in sodium acetate buffer (pH 5.0) were washed with the same bufffer; the arrow indicates the starting buffer containing 20 m*M* sodium phosphate.

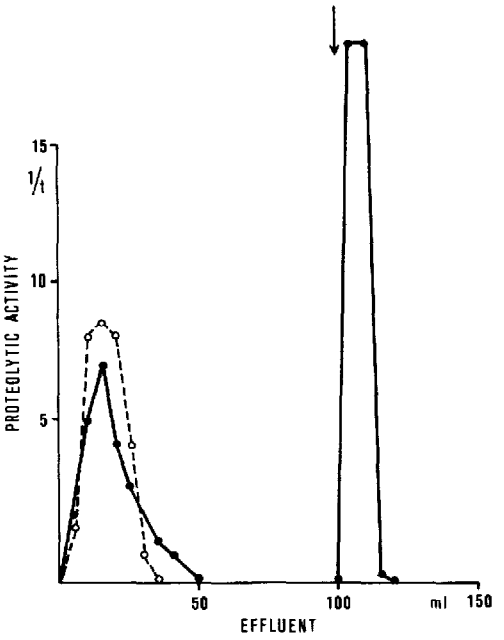

Fig. 4. Chromatography of human gastric juice before (solid line) and after (dashed line) dephosphorylation with acid phosphatase on an IDA-ferric Ultrogel column. Samples in sodium acetate buffer (pH 5.0) were washed with the same buffer; the arrow indicates the starting buffer containing 20 m*M* sodium phosphate.

man gastric juice to the extent of 10–60% and from the extract of gastric mucosa to the extent of 30–60%. While 60% of pepsinogens were adsorbed in all seven patients with gastric carcinoma only 30% of pepsinogens were adsorbed in five patients out of seven tested with ulcer disease, and 45% of pepsinogens in the other two patients. It may be suggested that the amount of enzymes may correlate with genetic type and gastric disease.

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