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Note

Separation of human pepsins in gastric juice by high-performance ion-exchange chromatography

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Separation of the pepsin components of human gastric juice, as classified by Etherington and Taylor [1] has been previously achieved using conventional methods of protein purification, including repeated chromatography on DEAE cellulose [2] and preparative agar gel electrophoresis [3]. These methods are time-consuming with the chromatography extending over several days and large volumes of eluent being required for the milligramme quantities of protein recovered. We have, therefore, investigated the use of an anion-exchange column, using a conventional high-performance liquid chromatographic (HPLC) system.

EXPERIMENTAL

Chemicals and reagents

The chemicals used were of Analar grade (BDH, Poole, U.K.) unless stated otherwise. Agarose was obtained from Oxoid (London, U.K.), naphthalene black, bovine haemoglobin and swine pepsin were obtained from Sigma (Poole, U.K.).

Apparatus

The ion-exchange column was DEAE 5PW (TSK, Tokyo, Japan) 7.5×0.75 cm I.D., 10 μm particle size. A guard column packed with TSK guard gel DEAE 5PW was used. The HPLC gradient system comprised two constametric 11G pumps and a dynamic mixer (high pressure) (LDC, Stone, U.K.). The eluent was monitored at 280 nm using a variable-wavelength detector set at 0.1 a.u.f.s. (SpectroMonitor III, LDC). The samples were injected automatically using a Gilson 231 autosampler (Anachem, Bedford, U.K.) through a 250-μl loop.

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Methods

Chromatography. A linear gradient between 0 and 1.0 *M* sodium chloride in 0.1 *M* sodium acetate buffer, pH 4.1, containing 1.0 m*M* EDTA was used over 30 min with a 4-min hold at 1.0 *M* sodium chloride before resetting to the initial conditions. The flow-rate was optimised at 1.0 ml/min and gave a back-pressure of less than 28.6 bar. The buffers used were filtered through a 0.45-μm Millipore filter under vacuum and were degassed freshly before use.

Samples. Human gastric juice was obtained from patients undergoing routine gastric function tests with pentagastrin as the stimulant. The samples used for chromatography were dialysed against the starting buffer (0.1 *M* sodium acetate, pH 4.1), centrifuged at 700 *g*, and the clear supernatant (250 μl) was injected as necessary. Authentic pepsins 1 and 3 were prepared by the method of Roberts and Taylor [2].

Agar gel electrophoresis. Agar gel electrophoresis [3] was carried out at pH 5.0 using the Panagel electrophoresis unit (Millipore, London, U.K.) for 50 min at 150 V and 40 mA. The stain was 0.1% naphthalene black in methanol–water (1:1, v/v).

Proteolytic activity. Proteolytic activity in the chromatographic fractions was measured by the method of Gray and Billings [4].

RESULTS

Fig. 1 is a chromatogram of swine pepsin. Two peaks, only partially resolved, containing proteolytic activity are observed. Pure human pepsin 3, which is the principal human pepsin, chromatographs, however, as a single symmetrical peak of proteolytic activity. The concentrations of Cl^- ions required to elute swine pepsin and pepsin 3 were similar, being 0.35–0.40 *M* and 0.30–0.35 *M*, respectively. In two recovery experiments, using approximately 100 μg of human pepsin 3, 100 and 110% of the initial proteolytic activity was obtained after chromatography.

Purified human pepsin 1 (the peptic ulcer-associated pepsin), evidenced by a single zone of proteolytic activity on agar gel electrophoresis, elutes as a series of small unresolved peaks (Fig. 2) on the ascending limb of a large peak, the descending limb of which is smoothly well defined. All show proteolytic activity. The Cl^- concentration for elution extended over 0.30–0.65 *M*.

Human gastric juice (Fig. 3) gave two separate peaks (1 and 2a) of proteolytic activity corresponding to the individual enzymes, pepsins 3 and 5, respectively (Fig. 4). When human gastric juice was chromatographed with and without added swine pepsin, the two peaks of swine peptic activity were resolved from pepsin 3 and eluted between pepsin 1 and pepsin 3 similar to their mobility on agar gel electrophoresis [2]. A series of gastric juices from a pentagastrin stimulation test were applied to the column and it was possible from the elution profile to calculate the ratio of the amount of pepsin 3 to pepsin 5 (Table I).

In all the experiments with gastric juice, pepsin 1 was not eluted from the column. High-performance ion-exchange chromatography (HPIEC) has also

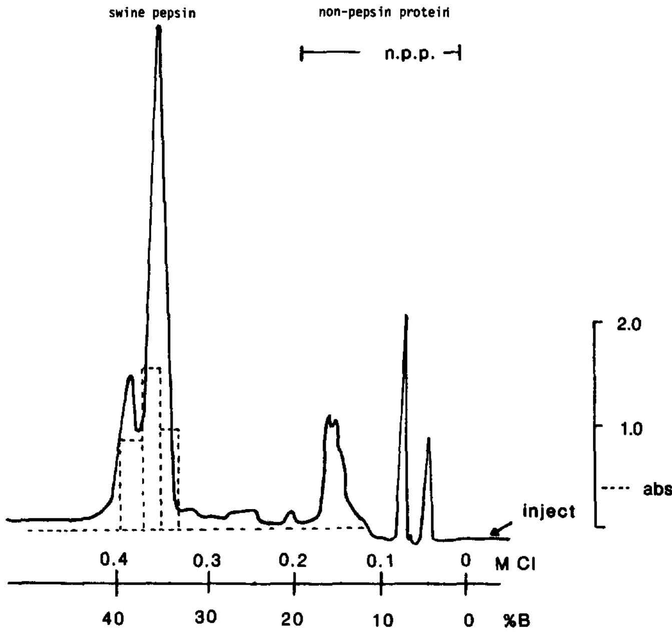

Fig. 1. Chromatographic tracing of pure swine pepsin during HPIEC. A 250-μg amount was injected on column. Swine pepsin shows two enzymatically active components. (—) Protein at 280 nm; (---) proteolytic activity of pooled fractions of the chromatogram, expressed as the change in absorbance of the substrate at 605 nm.

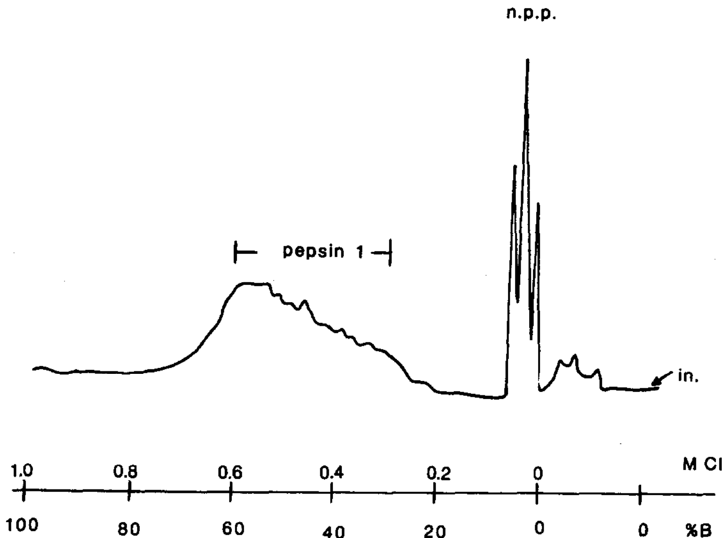

Fig. 2. Chromatographic tracing of pure human pepsin 1 during HPIEC. Approximately 20 μg of pepsin 1 were injected on the column. (—) Protein at 280 nm; n.p.p. = non-pepsin protein.

revealed a small peak of peptic activity, designated 2 in Table I, which migrates as a pepsin 3 on agar gel electrophoresis (Fig. 4).

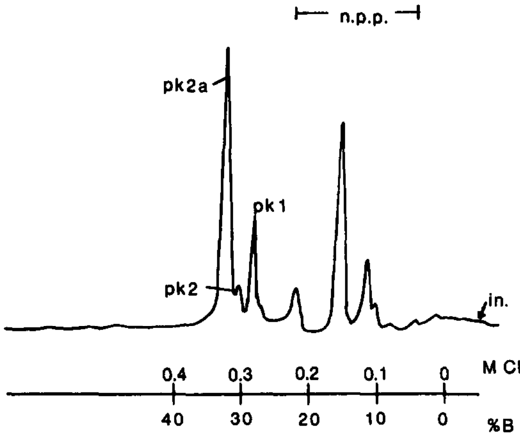

Fig. 3. Chromatographic tracing of the pepsins of human gastric juice during HPIEC. Peaks 1, 2 and 2a possess proteolytic activity. (—) Protein at 280 nm; n.p.p. = non-pepsin protein.

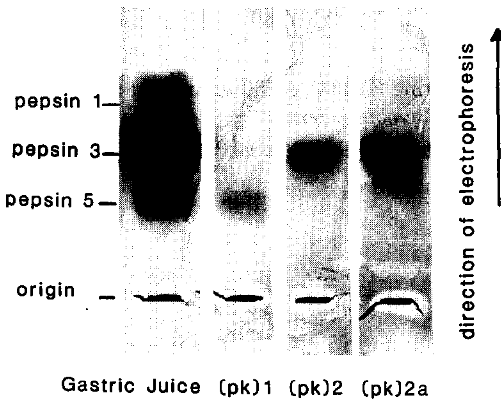

Fig. 4. Agar gel electrophoresis of the peaks 1, 2, and 2a from Fig. 4. Peak 1 consists of pepsin 5, peak 2 of pepsin 3 and peak 2a is the main peak of pepsin 3.

DISCUSSION

We have been able to demonstrate for the first time that the individual pepsins, such as pepsin 3 and swine pepsin, elute on HPIEC under very similar conditions to their separation on DEAE cellulose. Furthermore, the direct application of human gastric juice to HPIEC can resolve the major pepsins 3 and 5, which has always been difficult by conventional chromatography [2], requiring repeated chromatography on DEAE cellulose.

The relative amounts of pepsin 5 to pepsin 3 for the basal and first two post-pentagastrin-stimulated gastric juices agree with ratios reported previously using pepstatin-resistant activity measurements [5,6] and rocket immunoelectrophoresis [7]. The marked fall-off in the pepsin 5 content in samples 3 and 4 after pentagastrin is of interest and was not found previously [6]. The present and

TABLE I

RATIO OF PEPSINS 3 TO 5 FROM A PENTAGASTRIN TEST

The relative areas were calculated excluding pepsin 1; peak 2 is the proteolytic peak eluting between 3 and 5.

Juice	Volume (ml/10 min)	pH	Relative area			Ratio 3 to 5
			3	5	Peak 2	
Resting	73	3.78	66	32	2	2.1:1
Basal						
0–10 min	41	2.85	76	23	1	3.3:1
10–20 min	18	3.97	65	29	7	2.2:1
Post-pentagastrin						
0–10 min	60	1.79	82	14	4	5.9:1
10–20 min	88	1.26	90	10	–	9.0:1
20–30 min	89	1.25	100	<1	<1	
30–40 min	93	1.38	100	<1	<1	

previous reports only involved single subjects so that larger numbers need to be studied following pentagastrin stimulation.

The poor detection and measurement of pepsin 1, the pepsin associated with peptic ulcer disease [8], has been disappointing, for the enzyme was eluted over a rather broad range of chloride concentration. No defined peak of pepsin 1 was observed with gastric juice samples. Clearly further modifications of the gradient need to be investigated to improve the detection of pepsin 1. The possibility arises from the chromatography, that the enzyme is a heterogeneous mixture of very similar components although migrating as a single band on agar gel electrophoresis. Ryle and Foltmann [9] have recently made a similar suggestion after purification of pepsin 1 using DEAE-Sephadex; they suggested that the micro-heterogeneity was due to varying degrees of carbohydrate substitution on a common pepsin–protein backbone.

Thus it is now possible, using the described chromatographic procedure, not only to ascertain the composition of pepsins 3 and 5 in gastric juice but to prepare the individual pepsins more speedily.

Finally, attention should be drawn to the observation that swine pepsin A, unexpectedly, contains two components using our technique; this enzyme has been the subject of a number of, apparently definitive, structural studies and it would be of interest to know if the two components differ in amino acid composition or phosphate content or, perhaps, carbohydrate content.

CONCLUSIONS

An HPIEC system has been developed for the separation of pepsins. Individual human pepsins 1, 3 and 5 are eluted at specific Cl^- concentrations at pH 4.1. Separation of pepsins 3 and 5 from gastric juice can be achieved readily. Pepsin

1 in gastric juice appears not to elute under the conditions outlined. HPIEC thus presents a simple and rapid system for the separation and quantitation of pepsins 3 and 5 in human gastric juice.

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