*Journal of Chromatography*, 419 (1987) 323–328
*Biomedical Applications*
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 3701

## Note

# Isolation and characterization of a protein detected in inflamed human gastric mucosa

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(First received August 11th, 1986; revised manuscript received March 16th, 1987)

There is considerable current interest in the metabolic profiles of human tissues and fluids, including human gastric mucosa. The determination of the occurrence and the number of gastric proteases is of great diagnostic importance in various diseases. The mechanism of protein hydrolysis in the stomach has not yet been fully elucidated. The published papers concerning this problem are not always in agreement with each other and, moreover, are controversial [1].

The extract of human gastric mucosa is known to contain eight proteolytic fractions separable on agar gel electrophoresis. The first five are pepsinogens of the first group (pepsinogens A, EC 3.4.23.1), the next two are the two pepsinogens of the second group (pepsinogens C, EC 3.4.23.3) and the last fraction is the non-pepsin protease known as the slow-moving protease [2,3]. The number and the concentration of the individual fractions depend on the genetic context and the type of disease involved [4–7].

We have studied the occurrence of individual gastric proteases in the homogenate of gastric mucosa. An additional dark band was observed in some cases on agar gel electrophoresis in addition to the light-coloured bands. To our knowledge, this feature has not been previously reported, and it may be of interest in some gastric diseases.

0378-4347/87/$03.50 

## EXPERIMENTAL

### *Samples*

The extracts of human gastric mucosa were obtained from resected parts of stomach of patients suffering from gastric and duodenal ulcers and gastric carcinoma. Resected parts of the stomach were either deep-frozen (at −20°C) or processed immediately. They were washed with 0.1 *M* phosphate buffer (pH 7.3), then divided into several transversal strips. A part of each sample was taken for histological examination. Dissected mucosa was homogenized in 0.1 *M* phosphate buffer (pH 7.3) (4 ml per 1 g of tissue). The supernatant was separated by centrifugation at 16 000 *g* and analysed.

### *Reagents and chemicals*

Pepsin was from Léčiva (Prague, Czechoslovakia), bovine serum albumin, haemoglobin, ovalbumin and acrylamide were obtained from Serva (Heidelberg, F.R.G.), glucose oxidase from TS (Munich, F.R.G.), DEAE-cellulose from Whatman (Maidstone, U.K.), Sephadex G-100 from Pharmacia (Uppsala, Sweden) and agar from Difco (Detroit, MI, U.S.A.).

### *Electrophoresis*

Agar gel electrophoresis was carried out in 1.8% agar gel in 0.07 *M* Tris–glycine–veronal–HCl buffer (pH 8.3) at a potential gradient of 30 V/cm [8]. The proteolytic activity in the gel was detected using 0.6% haemoglobin at pH 2. The proteins were stained with Amidoblack. Polyacrylamide gel electrophoresis was carried out in 7.5% gel in Tris–EDTA–borate buffer (pH 8.3) at a gradient of 20 V/cm [9]. The protein concentration was monitored by UV absorbance at 280 nm and by the method of Lowry et al. [10]. After acrylamide gel electrophoresis the glycides were detected using Schiff's reagent for glycoproteins [11].

Protease activity was determined as milk-clotting activity [12] and by method of Anson and Mirsky [13]. Inhibition activity was estimated according to Uriel and Berges [14]. The hydrolysis of protein was determined after agar electrophoresis. The agar gel was treated with haemoglobin and then was exposed to a 3 m*M* solution of pepsin or trypsin for 1 h at 37°C, fixed with trichloroacetic acid (TCA) and stained with Amidoblack. The isoelectric point was determined by measurement of pH after dialysis of protein against distilled water for 72 h and by measurement of the pH of the peak of the protein after chromatography on Sephadex G-25 in distilled water. Immunological examination was made by IDP set from Sevac (Prague, Czechoslovakia), and anti IgG, anti IgA and anti IgM by methods of radial immunodiffusion. The amino acid composition was determined using an amino acid analyser after hydrolysis by 6 *M* hydrochloric acid at 110°C under vacuum for 70 h (Table I). The molecular weight (MW) was determined in two ways: (a) by gel chromatography on Sephadex G-100 according to Andrews [15] in 0.05 *M* sodium phosphate and 0.1 *M* potassium chloride at pH 7.3; the standards used were cytochrome C (MW 12 000), chymotrypsin (MW 23 000), ovalbumin (MW 43 000), bovine albumin (MW 68 000) and glucose

TABLE I

AMINO ACID COMPOSITION OF ISOLATED PROTEIN

| Amino acid | Number of residues per molecule of protein | Amino acid | Number of residues per molecule of protein |
|---|---|---|---|
| Asp | 36 | Met | 3 |
| Thr | 22 | Ileu | 7 |
| Ser | 22 | Leu | 4 |
| Glu | 51 | Tyr | 8 |
| Pro | 20 | Phe | 15 |
| Gly | 26 | His | 8 |
| Ala | 61 | Lys | 34 |
| Half-Cys | 20 | Arg | 13 |
| Val | 30 | | |

oxidase (MW 156 000); (b) by polyacrylamide gel electrophoresis (PAGE) according to Weber and Osborn [16], using the same standards.

*Isolation of protein*

The extract of human gastric mucosa (300 ml) was applied to a column (500×40 mm I.D.) of DEAE-cellulose equilibrated with 0.2 *M* sodium phosphate (pH 5.9). The column was washed with the same buffer. Elution was performed with the same buffer with a gradient of sodium chloride (0–1 *M*). Each fraction (5 ml) was examined for protein concentration and proteolytic activity (Fig. 1). Protein-containing fractions were dialysed, lyophilized and analysed by agar gel electrophoresis. The dark band contained only the third peak of protein eluted from the column. This fraction had no proteolytic activity, and on PAGE only one large peak with two weak bands appeared. Three peaks of this fraction were also found on Sephadex G-100 chromatography.

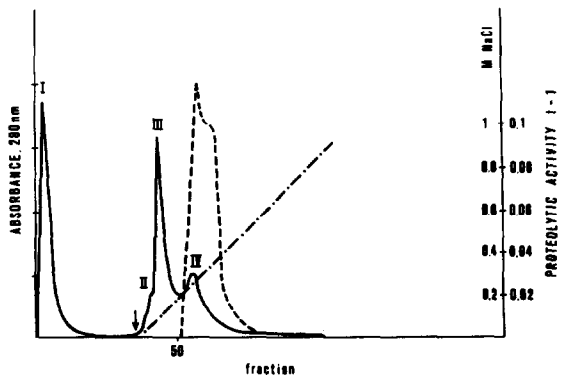


Fig. 1. Isolation of protein on DEAE-cellulose, showing absorbance at 280 nm (solid line), proteolytic activity (dashed line) and concentration of sodium chloride (dash-dotted line). Samples in 0.02 *M* sodium phosphate (pH 5.9), washed with the same buffer; arrow indicates the starting buffer containing a gradient of sodium chloride (0–1 *M*).

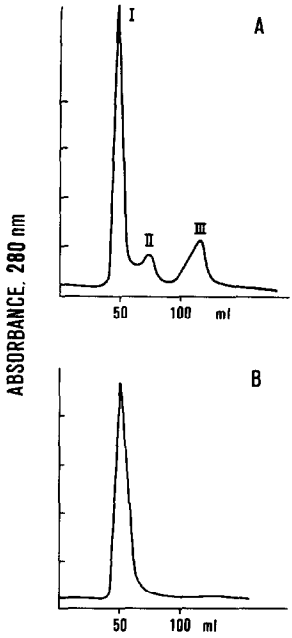


Fig. 2. Purification of fraction III on Sephadex G-100. (A) Purification; (B) re-chromatography of peak I. Samples in 0.05 *M* sodium phosphate with 0.1 *M* potassium chloride (pH 7.5); elution with the same buffer.

*Purification of non-proteolytic-enzyme-containing fraction III*

Fraction III from the DEAE-cellulose column was dialysed, and after lyophilization it was dissolved in 0.05 *M* sodium phosphate with 0.1 *M* potassium chloride (pH 7.5). This sample was loaded on a Sephadex G-100 column (500×20 mm I.D.) equilibrated with 0.05 *M* sodium phosphate with 0.1 *M* potassium chloride (pH 7.5). The elution was performed with the same buffer. Three protein peaks were eluted (Fig. 2A). The dark band contained only the first and the largest peak of protein.

## RESULTS AND DISCUSSION

A dark band was observed on zymograms of human gastric mucosa homogenates, appearing only in cases where gastritis was found histologically. This material was isolated by combined chromatography on DEAE-cellulose and Sephadex G-100. The protein obtained showed a single band on PAGE. Only one peak was eluted on re-chromatography on Sephadex G-100 (Fig. 2B). This was shown to be a protein with an MW of 52 000–57 000 and an isoelectric point (p*I*) of 6.7. According to the reaction with Schiff's reagent this protein did not contain any glycosides. The protein did not have inhibitory activity, nor did it react with antibody against human immunoglobulins. The dark band on agar gel electrophoresis did not change after treatment by pepsin or trypsin solution (Fig. 3), similarly to protein of human serum. This protein appeared only in those extracts

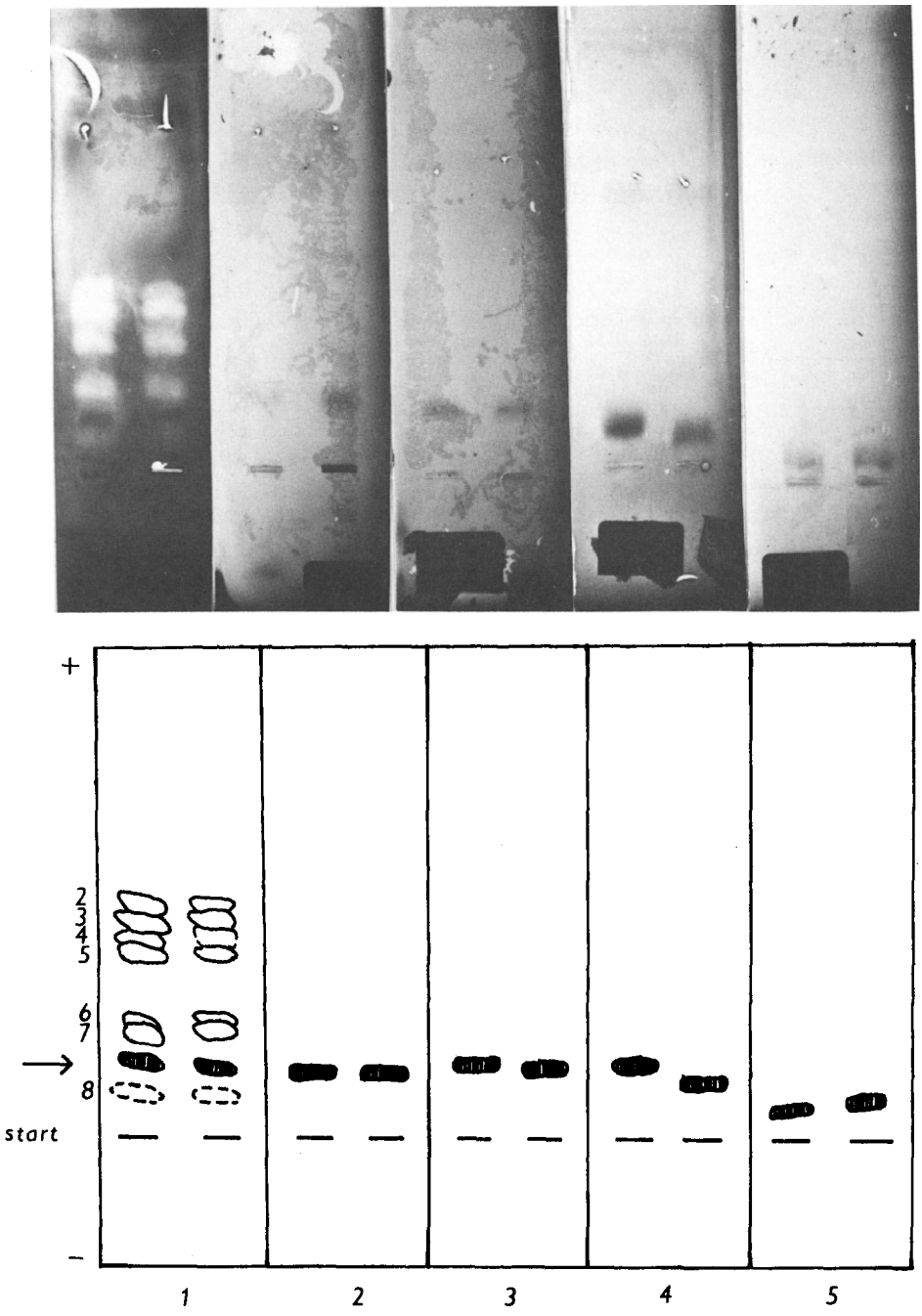


Fig. 3. Agar gel electrophoresis detected for proteolytic activity. (1) Two types of human gastric mucosa; (2) the same after pepsin digestion; (3) the same after trypsin digestion; (4) concentrated human serum and homogenate of human gastric mucosa after pepsin digestion; (5) human serum albumin and homogenate of human gastric mucosa after pepsin digestion.

of human gastric mucosa where significant chronic gastritis was observed. Sample of stomach where gastritis was absent had no dark band at all. It was detected in 15 of 40 patients with duodenal ulcers, in 4 of 21 patients with gastric ulcers and in 17 of 36 patients with gastric carcinoma.

This protein could be one of the proteins of human serum (eventually partly hydrolysed). It could be diffused into the mucosa during inflammation.

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