Journal of Chromatography, 376 (1986) 409—412
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2964

SEPARATION OF HUMAN PEPSIN AND GASTRICSIN BY AFFINITY CHROMATOGRAPHY WITH AN IMMOBILIZED SYNTHETIC INHIBITOR

Z. KUČEROVÁ *

Department of Pathological Physiology, Faculty of Medicine, Charles University, U nemocnice 5, 128 53, Prague 2 (Czechoslovakia)

J. POHL

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Flemingovo 2, 166 10 Prague 6 (Czechoslovakia)

and

L. KORBOVÁ

Department of Pathological Physiology, Faculty of Medicine, Charles University, U nemocnice 5, 128 53, Prague 2 (Czechoslovakia)

SUMMARY

Pepsin and gastricsin from human gastric juice were separated by affinity chromatography on Sepharose 4B containing the immobilized synthetic inhibitor of aspartic proteinases, Val-D-Leu-Pro-Phe-Phe-Val-D-Leu. These enzymes were bound to the support at low pH, and gastricsin was released at the same pH with buffer containing 20% dioxan. Pepsin was not released under these conditions, but was eluted at higher pH with buffer also containing 20% dioxan. To obtain perfect separations, it is recommended to use diluted samples. Proteinases from the homogenate of human gastric mucosa are isolated on DEAE-cellulose before separation by affinity chromatography. Pepsin and gastricsin from human gastric juice and human gastric mucosa separated on DEAE-cellulose and isolated by affinity chromatography, were electrophoretically pure.

INTRODUCTION

Human gastric juice and extract of human gastric mucosa contain two main groups of aspartic proteinases [1], pepsin (EC 3.4.23.1) and gastricsin (EC 3.4.23.3), which differ by their structural, kinetic and immunological characteristics. Pepsin and gastricsin are considered difficult to separate by ion-exchange chromatography, even in combination with immunoadsorption affinity chromatography [2—4]. Recently, we have published a single-step

0378-4347/86/$03.50

isolation procedure for aspartic proteinases from various sources by affinity chromatography on Sepharose [5] containing the immobilized synthetic inhibitor of aspartic proteinases, Val-D-Leu-Pro-Phe-Phe-Val-D-Leu. This peptide is a competitive inhibitor of aspartic proteinases. A significant difference in K_i values in solution for pepsin and gastricsin, i.e. $6 \cdot 10^{-7}$ and $1 \cdot 10^{-4}$ *M*, respectively, was exploited for separation of the two enzymes on this affinity support.

In this paper, a rapid single-step separation of pepsin from gastricsin from crude human gastric juice and prepurified human gastric mucosa extract is presented.

EXPERIMENTAL

Sepharose 4B from Pharmacia Fine Chemicals (Uppsala, Sweden), bovine haemoglobin and acrylamide from Serva (Heidelberg, F.R.G.) and agar from Difco (Detroit, MI, U.S.A.) were used. The inhibitor—Sepharose was prepared as described previously [5]. Human gastric juice was obtained from patients of the 2nd Department of Internal Medicine (Prague, Czechoslovakia). Extract of human gastric mucosa was prepared as described previously [6]. The activity of proteinases was determined by milk-clotting activity [7]. Electrophoresis on agar was carried out in 1.5% agar and 0.025 *M* sodium acetate buffer (pH 5) at a potential gradient of 20 V/cm [8]. The detection of proteolytic activity on zymograms was effected by 0.6% haemoglobin at pH 2.

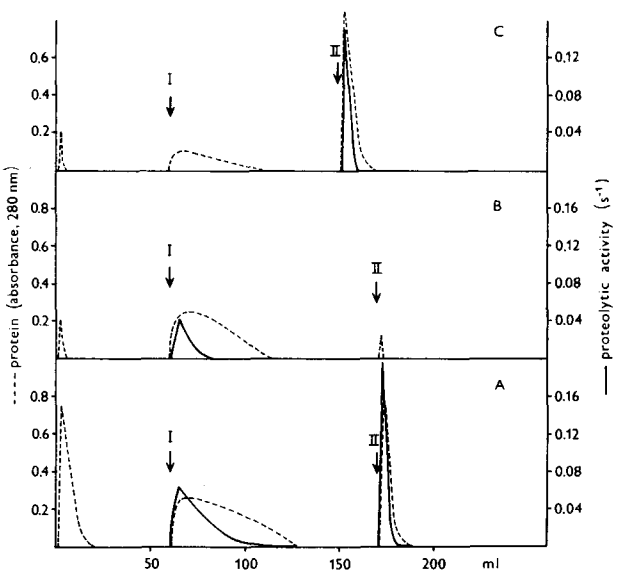

Fig. 1. Affinity chromatography of pepsin and gastricsin from human gastric juice. (A) Isolation; (B) rechromatography of peak I (gastricsin); (C) rechromatography of peak II (pepsin). Samples in 0.1 *M* sodium acetate buffer (pH 3.5) washed with the same buffer; arrow I, elution with the starting buffer containing 20% dioxan; arrow II, elution with 0.05 *M* sodium phosphate buffer (pH 6.2) containing 20% dioxan.

Preparation of the sample

Fresh human gastric juice was adjusted to pH 3.5 by sodium acetate and centrifuged at 1000 *g* for 30 min. The supernatant was used. Extract of human gastric mucosa was prepurified as described [4]. Zymogens were activated at pH 2 for 10 min at 25°C, and the pH was adjusted to 3.5 with excess of 0.1 *M* sodium acetate buffer (pH 3.5).

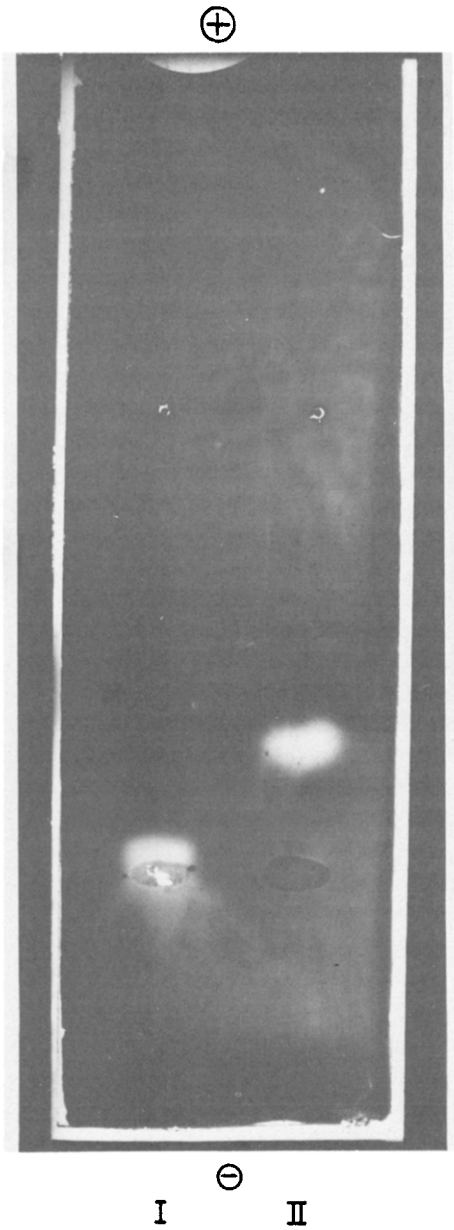

Fig. 2. Zymogram of agar gel electrophoresis of isolated pepsin and gastricsin: I, gastricsin; II, pepsin. 1.5% Agar; 0.025 *M* sodium acetate buffer (pH 5.0) at an elution gradient of 20 V/cm.

RESULTS AND DISCUSSION

Samples of human gastric juice (2 ml) were applied to a column (8 × 0.6 cm I.D.) of inhibitor—Sepharose equilibrated with 0.1 *M* sodium acetate buffer (pH 3.5). The column was washed with the same buffer. Elution of gastricsin was effected by the starting buffer containing 20% dioxan. Under these conditions, human pepsin remains bound to the column and is effectively eluted by the 0.05 *M* phosphate buffer (pH 6.2) containing 20% dioxan. The same behaviour was observed on rechromatography of purified pepsin and gastricsin on the same column (Fig. 1). No cross-contamination of the isolated proteinases was shown on agar electrophoretic zymograms (Fig. 2). To obtain perfect separations, it is recommended to use diluted samples.

Samples of human gastric mucosa cannot be separated by single-step separation. Proteinase from extract of human gastric mucosa is isolated on DEAE-cellulose before separation by affinity chromatography. Isolation by chromatography on Sephadex G-100 is insufficient. Affinity chromatography of a prepurified sample (2 ml) of human gastric mucosa extract was the same as described above for gastric juice.

Pepsin and gastricsin have very similar physicochemical as well as enzymatic characteristics [9]. So far, these enzymes were separated by lengthy procedures involving gel-permeation chromatography, anion-exchange chromatography or a combination of either with immunoadsorption. These methods are not applicable to clinical uses. The presented method is very simple and can be used both for preparative and, after adaptation of this method, to high-performance affinity chromatography for analytical purposes.

REFERENCES

1 I.M. Samloff, Gastroenterology, 57 (1969) 659.
2 J. Tang and K.I. Tang, J. Biol. Chem., 238 (1963) 606.
3 S. Matzku and W. Rapp, Biochim. Biophys. Acta, 446 (1976) 30.
4 B. Foltmann and A.L. Jensen, Eur. J. Biochem., 128 (1982) 63.
5 J. Pohl, M. Zaoral, A. Jindra, Jr. and V. Kostka, Anal. Biochem., 139 (1984) 265.
6 Z. Kučerová, L. Korbová, J. Čížková and J. Kohout, in V. Kostka (Editor), Aspartic Proteinases and their Inhibitors, Walter de Gruyter, Berlin, 1985, p. 525.
7 B. Foltmann, Methods Enzymol., 19 (1970) 421.
8 D.J. Etherington and W.H. Taylor, Biochem. J., 118 (1970) 587.
9 B. Foltmann, Essays Biochem., 17 (1981) 52.