Journal of Chromatography, 315 (1984) 478–480
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,185

Note

Isolation of trypsin by column chromatography on tea particles

IVO ŠAFAŘÍK
Department of Biochemistry and Microbiology, Institute of Chemical Technology, Suchbátarova 5, 166 28 Prague (Czechoslovakia)
(Received August 29th, 1984)

Because of autoproteolysis, proteolytic enzymes are usually isolated with low yields when a multi-step isolation procedure is used; the yield can be increased when one-step techniques are used for their isolation and purification[1].

Previous papers have described some one-step chromatographic purification techniques for the rapid isolation of various proteolytic enzymes. Thus, thermally modified casein was used for the isolation of trypsin[2]; the same enzyme was isolated on casein precipitated with trichloroacetic acid[3]. Agar was used for the isolation of trypsin[4], chymotrypsin[5], pancreatic proteases[4] and microbial proteases[6]. Trypsin[7] was also successfully isolated by column chromatography on sawdust.

Some other inexpensive materials can also be used for the rapid isolation of various proteases. It is shown in this paper that tea particles are suitable for the isolation of trypsin, which was used as a model protease.

EXPERIMENTAL

Materials

Tea (a mixture of some Indian varieties) was obtained from a local shop. Azocasein was prepared in the laboratory[8]. Trypsin was from Léčiva (Czechoslovakia), enzyme casein hydrolysate was from Imuna (Czechoslovakia). Sodium chloride and other common chemicals were from Lachema (Czechoslovakia).

Preparation of chromatographic sorbent

Tea was repeatedly suspended in boiling water until the brown substances were released. The prepared sorbent, suspended in water, was then packed into a glass column and further washed with water, 1 *M* sodium chloride solution, and water again, until the absorbance of the washings was lower than 0.01 at 280 nm in a 1-cm cuvette.

Chromatography of trypsin

A glass column (200 × 12 mm I.D.) filled with the washed sorbent to a height of 110 mm was used. After sample application, the ballast proteins were eluted with water. The adsorbed trypsin was eluted from the column with 1 *M* sodium chloride solution until no enzyme activity could be detected in the effluent. The flow-rates

0021-9673/84/$03.00 © 1984 Elsevier Science Publishers B.V.

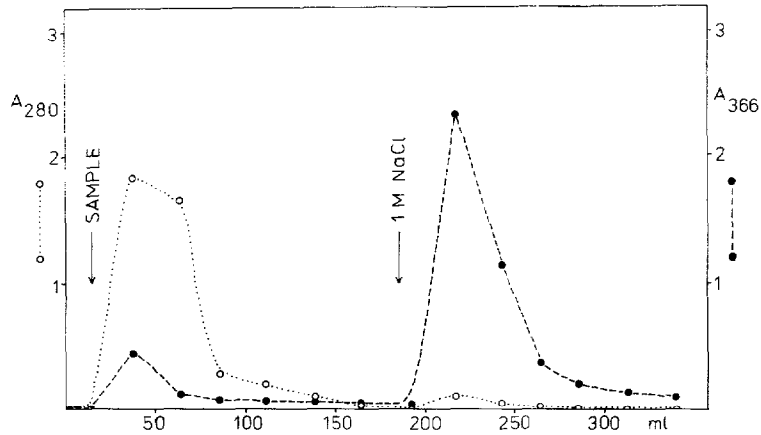

Fig. 1. Chromatography of 10 ml of a model mixture containing 10 mg of trypsin and 100 mg of enzyme casein hydrolysate on a 110 × 12 mm I.D. tea column. The column was washed with water and 1 *M* sodium chloride solution. The flow-rate was 1–2 ml/min. Dotted line, absorbance at 280 nm (protein content); dashed line, absorbance at 366 nm (trypsin activity).

ranged from 1 to 2 ml/min. The separations were carried out at laboratory temperatures.

Other procedures

The protein content in the eluted fractions was monitored spectrophotometrically at 280 nm, and the method of Warburg and Christian[9] was used for quantitative determinations.

The proteolytic activity in the eluted fractions was determined with azocasein as substrate, as described previously[2].

RESULTS AND DISCUSSION

In the first experiment, 10 ml of a model mixture containing 10 mg of trypsin and 100 mg of enzyme casein hydrolysate were used for chromatography. Fig. 1 shows the distribution of total proteins and the trypsin activity in the effluent. Of the trypsin activity applied, 17.5% was eluted with water, together with ballast proteins, and 65.5% was found in the first 50 ml of effluent after the elution conditions were changed; the recovery was 80.7% in the total volume of 150 ml. The degree of purification based on specific activity was 18.5-fold in a 80.7% yield.

To determine the capacity of the sorbent, 300 mg of trypsin in 40 ml of water were applied to the same column. After elution of non-bound trypsin and ballast proteins with water, the adsorbed trypsin was eluted with 1 *M* sodium chloride solution. The capacity was *ca.* 2 mg of pure trypsin per millilitre of the packed sorbent.

Probably some other proteolytic enzymes could be simply isolated using this sorbent, which is inexpensive and can be obtained in large amounts.

REFERENCES

1 C. R. Lowe and P. D. G. Dean, *Affinity Chromatography*, Wiley, Chichester, 1974.

2 I. Šafařík, *J. Chromatogr.*, 261 (1983) 138.
3 I. Šafařík, *J. Chromatogr.*, 284 (1984) 515.
4 I. Šafařík, *Biomed. Biochim. Acta*, 43 (1984) 1041.
5 I. Šafařík, Z. Laudová and B. Králová, *J. Chromatogr.*, 303 (1984) 283.
6 I. Šafařík, *J. Chromatogr.*, 298 (1984) 531.
7 I. Šafařík, *J. Chromatogr.*, 294 (1984) 504.
8 J. Charney and R. M. Tomarelli, *J. Biol. Chem.*, 171 (1947) 501.
9 O. Warburg and W. Christian, *Biochem. Z.*, 310 (1941) 384.