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Note

Purification of chymotrypsin and trypsin by column chromatography on agar gel particles

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Agar has successfully been used for the isolation and purification of human urine urokinase[1], trypsin[2], pancreatic proteases[2] and extracellular proteases produced by a strain of *Bacillus* sp. FB2 (ref. 3). In all cases, the powder form was employed for chromatography.

It is shown in this note that agar in the gel form can be also used for the chromatographic isolation of some proteolytic enzymes.

EXPERIMENTAL

Materials

Agar No. 3 was obtained from Oxoid (U.K.). Azocasein was prepared in the laboratory[4]. Chymotrypsin and trypsin were from Léčiva (Czechoslovakia), enzyme casein hydrolysate from Imuna (Czechoslovakia). Sodium chloride and other reagents were from Lachema (Czechoslovakia).

Preparation of chromatographic sorbent

Agar (3 g) was suspended in 100 ml of water and autoclaved at 121°C for 20 min. After cooling the resultant agar gel was cut with a knife into smaller portions and forced through a sieve (mesh dimension 1 × 1 mm) to form particles. The dimensions of these particles ranged from 1 × 1 × 1 to 1 × 1 × 3 mm. They were suspended in water and after decantation (removal of fines) were placed in a glass column. The sorbent was washed alternately with water and 1 *M* sodium chloride solution until the absorbance of the washings was lower than 0.01 at 280 nm in a 1-cm cuvette.

Chromatography of proteases

Glass columns (300 × 12 mm I.D.) filled to a height of 150 mm with washed sorbent were used throughout. After sample application the ballast proteins were eluted with water. The adsorbed proteases were eluted from the column with 1 *M* sodium chloride solution until no further enzyme activity could be detected in the effluent. The flow-rate was 1 ml/min. The separations were carried out at laboratory temperature.

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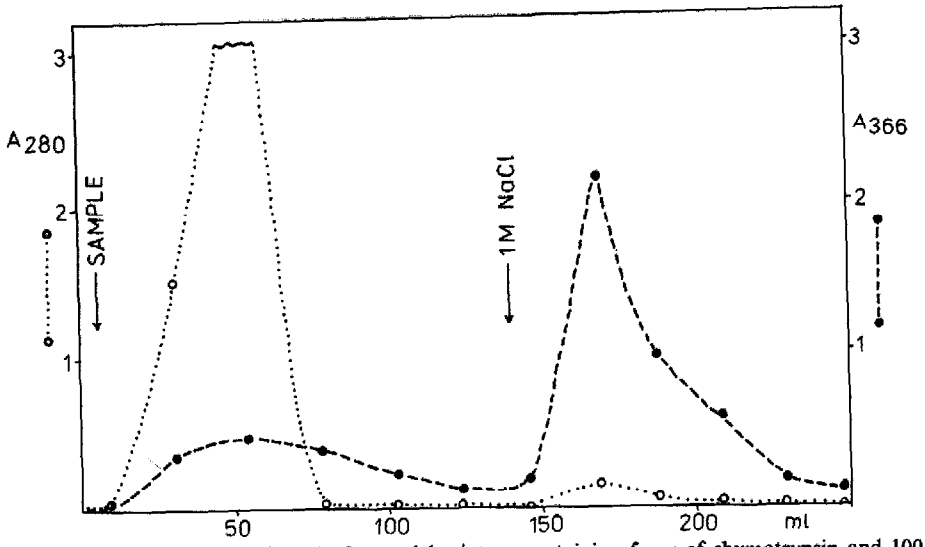

Fig. 1. Chromatography of 7 ml of a model mixture containing 5 mg of chymotrypsin and 100 mg of enzyme casein hydrolysate on a 150 × 12 mm I.D. column of agar gel particles. The column was washed with water and 1 *M* sodium chloride solution. The flow-rate was 1 ml/min. Absorbances: O · · · O, at 280 nm, protein content; ●---●, at 366 nm, chymotrypsin activity.

Other procedures

The protein content in the eluted fractions was monitored spectrophotometrically at 280 nm, and the Warburg and Christian[5] method was used for quantitations. The proteolytic activity in the eluted fractions was determined with azocasein as substrate, as described previously[6].

RESULTS AND DISCUSSION

In the first experiment, 7 ml of a model mixture containing 5 mg of chymotrypsin and 100 mg of enzyme casein hydrolysate were used for chromatography. Fig. 1 shows the distribution of total proteins and the chymotrypsin activity in the effluent. Of the chymotrypsin activity applied, 26.5% was eluted with water together with ballast proteins and 70.2% was eluted with 1 *M* sodium chloride solution in a total volume of 110 ml. The specific activity of chymotrypsin increased 15-fold after chromatography.

Similar results were obtained for a model sample containing trypsin.

The use of agar gel particles instead of agar powder may further reduce the cost of the chromatographic sorbent and the whole chromatographic separation.

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