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Note

Chromatography of trypsin on a sawdust column

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Microbial, animal and plant proteolytic enzymes can be isolated and purified using a variety of methods, of which various chromatographic procedures, including gel, ion-exchange and affinity chromatography, are the most frequently used[1–5].

Kobayashi *et al.*[6] successfully used a sawdust column for the isolation and purification of human urine urokinase. It is shown in this paper that sawdust can also be used for the isolation and purification of trypsin.

EXPERIMENTAL

Materials

Sawdust from pine wood was obtained from a local joiner's workshop. Azocasein was prepared in the laboratory[7]. Trypsin was purchased from Léčiva (Czechoslovakia), enzyme casein hydrolysate from Imuna (Czechoslovakia) and ammonium sulphate and other common chemicals from Lachema (Czechoslovakia).

Preparation of chromatographic sorbent

Sawdust with particles of diameter 0.5–2 mm was repeatedly washed with 4% sodium hydroxide solution, water, 3% hydrochloric acid and water until no further brown colour was released. The prepared sorbent, suspended in water, was then packed into a glass column and further washed with water, 1 *M* ammonium sulphate solution and water, until the absorbance of the washings was lower than 0.01 at 280 nm in a 1-cm cuvette.

Chromatography of trypsin

Glass columns (300 × 12 mm I.D.) filled with the washed sorbent to a height of 150–170 mm were used in all the experiments. After sample application, the ballast proteins were eluted with water. The adsorbed trypsin was eluted from the column with 1 *M* ammonium sulphate solution until no enzyme activity could be detected in the effluent. The flow-rates ranged from 1 to 2 ml/min. The separations were carried out at laboratory temperature.

Other procedures

The protein content in the eluted fractions was monitored spectrophotomet-

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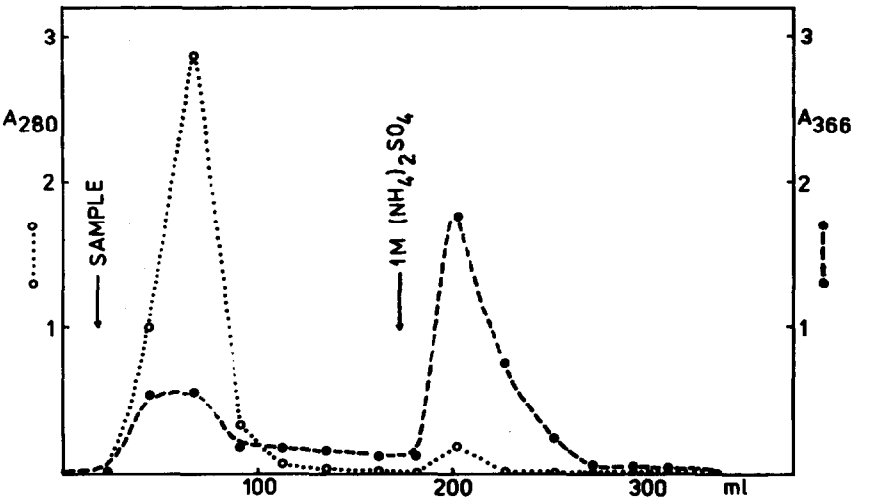

Fig. 1. Chromatography of 10 ml of a model mixture containing 10 mg of trypsin and 100 mg of enzyme casein hydrolysate on a 170 × 12 mm I.D. sawdust column. The column was washed with water and 1 *M* ammonium sulphate solution. The flow-rate was 1–2 ml/min. Dotted line, absorbance at 280 nm (protein content); dashed line, absorbance at 366 nm (trypsin activity).

rically at 280 nm, and the Warburg and Christian method was used for quantitative determinations[8].

The proteolytic activity in the eluted fractions was determined with azocasein as substrate, as described previously[9].

RESULTS

In the first experiment, 10 ml of a model mixture containing 100 mg of enzyme casein hydrolysate and 10 mg of trypsin were used for chromatography on 170 × 12 mm I.D. sawdust column. Fig. 1 shows the distribution of total proteins and the trypsin activity in the effluent. Of the trypsin activity applied, 35.4% was eluted with water together with ballast proteins and 47.1% was found in the first 45 ml of effluent after changing the elution conditions; the recovery was 53.3% in the total volume 150 ml. The degree of purification based on specific activity was 11.7-fold in a 53.3% yield.

Similar results were obtained when 10 mg of trypsin (an approximately 6-year-old preparation) in 10 ml of water were applied to an analogous sawdust column (150 × 12 mm I.D.). Of the trypsin activity applied, 24.9% was eluted with water together with the ballast proteins and 70.3% was eluted with 1 *M* ammonium sulphate solution in the total volume of 145 ml. The specific activity of trypsin had increased 1.7-fold after chromatography.

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* ammonium sulphate solution. The capacity was approximately 0.55 mg of pure trypsin per millilitre of the packed sorbent.

DISCUSSION

Chromatography on sawdust could be a promising technique for the isolation and purification of some biologically active macromolecules. In addition to trypsin, human urine urokinase[6] and one component of elastases of human pancreatic juice and pancreatic extract[10,11] were obtained in a highly purified state by chromatography on a sawdust column.

The cheapness of sawdust makes this technique promising for large-scale procedures.

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