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Note

Affinity chromatography of trypsin on casein precipitated with trichloroacetic acid

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Affinity chromatography occupies a unique place in separation technology as it is the only technique that permits the purification of almost any biomolecule on the basis of its biological function or individual chemical structure.

For proteases, several techniques based on reversible interactions between an appropriate ligand (substrate, substrate analogue, inhibitor, antibody) and the isolated protease(s) can be used[1–3]. The above ligands are usually immobilized to an insoluble support. To avoid the immobilization procedure, one can use directly an appropriate insoluble ligand, *e.g.*, substrate, for chromatography. Thus, Gallop *et al.*[4] used native collagen for the isolation of collagenase and trypsin was successfully isolated by chromatography on casein insolubilized by heating[5].

Another means of obtaining insoluble proteins for affinity chromatography of proteases is to precipitate suitable proteins from solution with an appropriate precipitating agent[6]. In this paper, the use of trichloroacetic acid for casein precipitation and the subsequent use of the precipitate for the isolation and purification of trypsin is described.

EXPERIMENTAL

Materials

Casein after Hammarsten and trichloroacetic acid were obtained from Reanal (Budapest, Hungary). Azocasein was prepared in the laboratory[7]. Trypsin was purchased from Léčiva (Czechoslovakia), enzyme casein hydrolysate from Imuna (Czechoslovakia) and sodium chloride and other common chemicals from Lachema (Brno, Czechoslovakia).

Preparation of affinity chromatographic sorbent

To a 4% solution of casein in 0.1 *M* disodium hydrogen orthophosphate a 10% solution of trichloroacetic acid was added until precipitation was complete. The precipitate was filtered, washed with water, dried at 90°C, then heated for 4 h at 180–190°C in a hot-air thermostat. The resulting material was ground in a mortar and sieved and the particles of diameter 0.3–0.5 mm were repeatedly suspended in water until no foaming due to the presence of liberated protein was observed. The

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sorbent suspended in water was then placed in a glass column and further washed alternately with water and 1 *M* sodium chloride solution until the absorbance of the washings was lower than 0.02 at 280 nm in a 1-cm cuvette.

Affinity chromatography of trypsin

Glass columns (300 × 12 mm I.D.) filled with washed sorbent to a height of 200 mm 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-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 spectrophotometrically at 280 nm, and the Warburg and Christian method[8] was used for quantitative determinations. The proteolytic activity in the eluted fractions was determined with azocasein as substrate, as described previously[5].

RESULTS AND DISCUSSION

In the first experiment, 2 ml of a model mixture containing 2 mg of trypsin and 24 mg of enzyme casein hydrolysate were used for chromatography. Fig. 1 shows the distribution of total proteins and the protease activity in the effluent. Of the protease activity applied, 36.2% was eluted with water together with ballast proteins

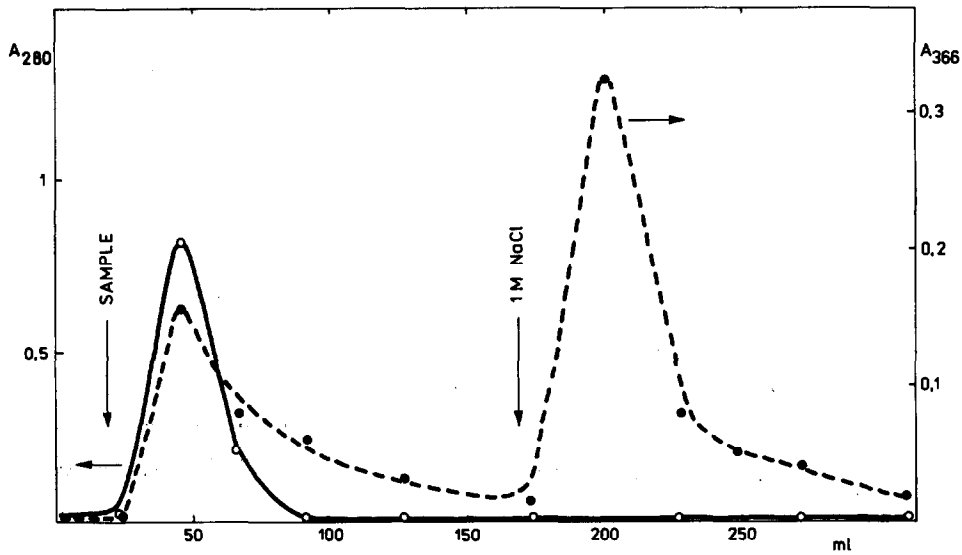

Fig. 1. Chromatography of 2 ml of a model mixture containing 2 mg of trypsin and 24 mg of enzyme casein hydrolysate on a 200 × 12 mm I.D. column of casein precipitated with trichloroacetic acid. The column was washed with water and 1 *M* sodium chloride solution. The flow-rate was 1–2 ml/min. Solid line, absorbance at 280 nm; broken line, absorbance at 366 nm (protease activity).

and 59.1% was found in the first 142 ml of effluent after changing the elution conditions. The specific activity of trypsin increased 19-fold after chromatography.

Similar results were obtained when 2 mg of trypsin in 4 ml of water were applied to the same column. Of the protease activity applied, 30.5% was eluted with water together with ballast proteins and 65.5% was eluted with 1 *M* sodium chloride solution in a total volume of 150 ml. The specific activity of trypsin increased 1.8-fold after chromatography.

To determine the capacity of the sorbent, 50 mg of trypsin in 25 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 approximately 0.1 mg of pure trypsin per millilitre of the packed sorbent.

Probably other proteins could be used for the preparation of sorbents in a similar way for the isolation of a variety of proteases.

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