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Note

Affinity chromatography of chymotrypsin (E.C. 3.4.21.1) on the potato trypsin inhibitor bound to bead cellulose by the benzoquinone method

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Different affinants were used for the affinity chromatography of chymotrypsin, *e.g.*, 4-phenylbutylamine[1], derivatives of D-tyrosine or D-tryptophan[2–4] and natural protease inhibitors[5,6]. Natural protease inhibitors are little influenced, if at all, by the support matrices and therefore are preferred in some instances.

The affinant is usually bound to the support by the cyanogen bromide method, and the supports most frequently used are agarose gels or modified dextran gels (Sephadex). Cellulose has been used as a support in some instances.

Previously bead cellulose has not been used as a support for affinity chromatography of proteases and the benzoquinone method has not been used for binding of the affinant. The use of both bead cellulose and the benzoquinone method is to be prefered for the industrial separation processes. The benzoquinone method avoids the use of highly toxic reagents such as cyanogen bromide. The good physical properties of bead cellulose[7,8] made high flow-rates in the column possible, thus enhancing the column efficiency.

In this work, the affinity sorbent was prepared by binding the trypsin inhibitor from potatoes to the bead cellulose by the benzoquinone method and its properties were studied.

EXPERIMENTAL

The protein concentration was determined according to the method of Hartree[9]. The protein concentration in the column effluent was measured by a UV monitor (Developmental Workshops of Czechoslovak Academy of Sciences, Prague, Czechoslovakia) at 280 nm. The proteolytic activity was measured by the method of Slavík and Smetana[10] and was expressed in casein units (C.U.); 1 C.U. is the amount of the enzyme that liberates 1 mg of peptides soluble in 0.1 *M* trichloroacetic acid from a 2% solution of casein after 60 min at 30°C and the optimal pH for the protease.

The bead cellulose was activated with the use of benzoquinone by the method described by Brandt *et al.*[11], except that ethanol was used instead of dimethylformamide as a solvent. The bead cellulose (particle diameter range 0.3–0.5 mm, mean 0.42 mm) was obtained from Spolek pro chem. a hutni výrobu, Ústí n.L., Czechoslovakia. The water regain of this support was 7.06 g/g, the coefficient *K* (see ref. 12; from

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the equation $u = K\,\Delta p/L$, where u = linear flow-rate, Δp = pressure drop and L = bed height) was 10.6 and the sedimentation velocity was 0.28 cm/sec.

Chymotrypsin was obtained from Spofa (Prague, Czechoslovakia), with a specific activity of 4.61 C.U. per milligram of protein and the protein content of 794 mg per gram of product. Trypsin inhibitor from potatoes was prepared as described by Ryan and Kassell[13]. Benzoquinone was prepared as described by Vliet[14].

RESULTS AND DISCUSSION

Trypsin inhibitor prepared from potatoes was bound to the bead cellulose in an amount of 200 mg per gram of the support. The yield of bound proteins was 56.2% (112.4 mg of protein was bound to each gram of support). The static capacity of the sorbent for the chymotrypsin (n_s)[15] was 106 mg per gram of sorbent and the operational capacity (n_0)[15] was 98.5 mg/g. The amount of total bound affinant could not be measured as the affinant was a crude product.

Affinity chromatography was performed in a 36 × 0.9 cm I.D. column a total amount of 1588 mg of protein being used for one run. The starting buffer was 0.1 *M* Tris–glycine (pH 8.01) and the non-proteolytic protein was eluted with this buffer. After the UV absorption had fallen to the starting value, the buffer was changed to

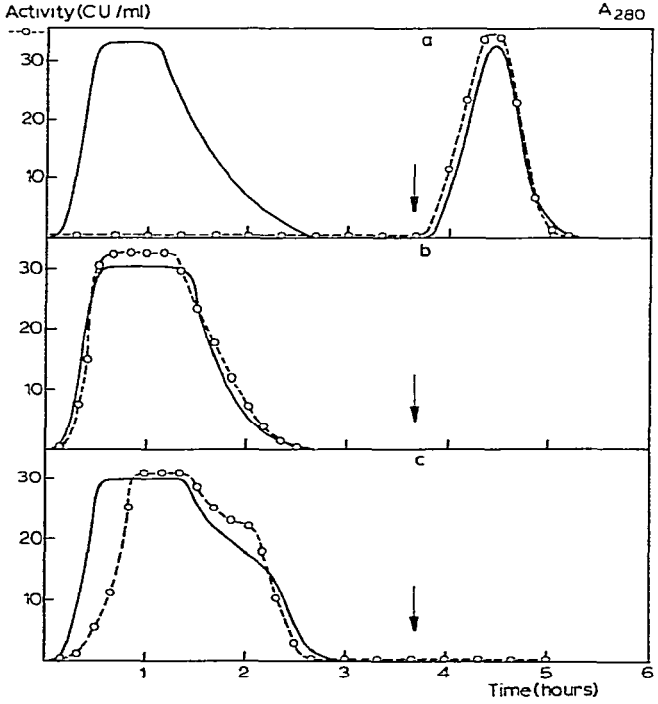

Fig. 1. Chromatography of chymotrypsin: (a) on trypsin inhibitor covalently bound to cellulose beads; (b) on unsubstituted cellulose beads; (c) on cellulose beads activated with benzoquinone in which the reactive groups are blocked with ethanolamine. Arrows indicate the change of starting buffer (0.1 *M* Tris–glycine, pH 8.01) for the elution buffer (0.1 *M* glycine–HCl, pH 2.07). The absorbance (solid lines) at 280 nm is given in arbitrary units, and the proteolytic activity (broken lines) in casein units per millilitre of effluent.

0.1 *M* glycine–hydrochloric acid (pH 2.07) and the chymotrypsin was eluted in a single peak containing 96.2% of the total proteolytic activity used. The specific activity of purified chymotrypsin was 22.4 C.U./mg, which is 4.86 times higher than the original value.

The results are shown in Fig. 1a. The same experimental conditions were used with the unsubstituted bead cellulose (Fig. 1b) and with the activated bead cellulose in which the reactive groups were blocked with ethanolamine (Fig. 1c). From the elution pattern it can be seen that the unsubstituted cellulose is ineffective in the chymotrypsin separation. All of the proteins added were eluted in a single peak. When using activated blocked bead cellulose, the chymotrypsin was partially separated from other proteins during the elution with the starting buffer, but the specific activity of this separated fraction was only 12% higher than the original value.

CONCLUSION

The results demonstrate the usefulness of the benzoquinone method with bead cellulose as a support in affinity chromatography. This method avoids the use of cyanogen bromide and will allow the industrial development of affinity sorption. The use of this method of affinant binding in connection with bead cellulose may be even more advantageous, taking into account the excellent physical properties of the support material.

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