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Note

Chromatographic procedure for preparation of a high-purity C 1 esterase inhibitor concentrate

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Wickerhauser et al. [1] have recently reported a method for preparation of a heat-treated, high-purity C 1 esterase inhibitor (C 1 INH) concentrate. These authors used batch ion-exchange adsorption of cryosupernatant plasma [2] followed by precipitation with polyethylene glycol and a final step purification by ammonium sulphate precipitation. Salt precipitation has the significant disadvantages of requiring centrifugation and extensive dialysis or diafiltration of the recovered fraction. As an alternative to salt precipation we have developed a simplified, chromatography-based procedure for final step purification of C 1 INH for use as a clinical concentrate.

EXPERIMENTAL

A 30-l pool of normal human factor IX-depleted plasma [3] was mixed with 6.0 g/l DEAE Sephadex A50 (Pharmacia, Uppsala, Sweden) for 45 min at room temperature. The DEAE Sephadex was recovered by sedimentation, washed with 500-ml aliquots of 0.1 *M* phosphate-buffered saline (0.025 *M* KH_2PO_4/Na_2HPO_4–0.075 *M* NaCl, pH 6.8) and the C 1 INH eluted with 300-ml aliquots of 1.0 *M* phosphate-buffered saline (0.2 *M* KH_2PO_4/Na_2HPO_4–0.8 *M* NaCl, pH 6.8). The eluted C 1 INH was desalted into 0.15 *M* sodium chloride using a 2.2-l column of Sephadex G25 (Pharmacia). PEG-4000 was added to the desalted eluate to a concentration of 20% (w/v), mixed for 1 h at room temperature and the supernatant recovered by centrifugation. The PEG-4000 supernatant was diluted 1:1 with sterile distilled water and adjusted to pH 5.5 with 0.1 *M* acetic acid. The solution was pumped onto a 2.3-l column (150 mm×140 mm I.D.) of CM-Se-

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pharose Fast Flow (Pharmacia) equilibrated in 0.050 *M* sodium acetate, pH 5.5. Unbound proteins were eluted by washing with starting buffer. Bound proteins were recovered by step gradient elution with starting buffer containing 0.1 *M* sodium chloride, starting buffer containing 0.2 *M* sodium chloride and starting buffer containing 1.0 *M* sodium chloride. C 1 INH was eluted with the starting buffer containing 0.2 *M* sodium chloride and collected, and the pH was immediately adjusted to 7.0 by the addition of solid Tris base. The product was buffer-exchanged into 0.01 *M* sodium citrate–0.13 *M* sodium chloride, pH 7.1, by diafiltration, concentrated to about 30 U/ml activity using ultrafiltration (Ultrasart, Sartorius, F.R.G.) and then sterile filtered, dispensed and lyophilised. The lyophilised product could be heat-treated at 80 °C for 24 h with only 10–15% loss of activity.

C 1 INH functional activity was measured using a modification [4] of a chromogenic peptide substrate method [5]. Rocket immunoelectrophoresis [6] was used to estimate C 1 INH, C2 and C4 antigen levels. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) [7], agarose electrophoresis [8] and immunoelectrophoresis [9] were used to assess the purity of individual fractions.

All chemicals and reagents used in this study were purchased from either Sigma (St. Louis, MO, U.S.A.) or Serva (Heidelberg, F.R.G.).

RESULTS

A summary of the analysis of C 1 INH purification from plasma is shown in Table I. The PEG precipitation step removed all detectable C2 and C4 with acceptable loss of C 1 INH. Detailed analysis of the CM Sepharose Fast Flow step is given in Table II and Fig. 1. The viscosity of the diluted PEG supernatant fraction limited the flow-rate during loading of the CM Sepharose to 100 cm/h. However, linear flow-rates of up to 200 cm/h could be used at the later stages of the elution sequence.

The CM-Sepharose step gave a 22-fold increase in the purity of C 1 INH with a step recovery of 70%. The volume of the C 1 INH fraction eluted by the 0.2 *M* sodium chloride buffer was generally about 0.15 times the CM-Sepharose load

TABLE I

ANALYSIS OF PURIFICATION OF C 1 INH FROM PLASMA

Fraction	Volume (l)	Total protein (mg/ml)	C 1 INH activity		C 1 INH specific activity (U/mg)	C 1 INH purification (×)
			U/ml	%		
Start plasma	30.0	63	1.0	100	0.016	–
DEAE eluent	3.3	25	3.5	38	0.14	9
PEG supernatant	3.8	11	2.2	28	0.20	13
CM eluent	1.2	1.1	4.8	19	4.36	272
Final concentrate	0.2	3.6	24	16	6.67	417

TABLE II

ANALYSIS OF CM-SEPHAROSE PURIFICATION STEP

Fraction	Volume (l)	Total protein		C 1 INH activity	
		mg/ml	%	U/ml	%
Load	7.6	5.4	100	1.0	100
Peak 1	9.6	2.6	61	0	0
Peak 2	3.1	2.6	20	0	0
Peak 3	1.2	1.1	3	4.8	76
Peak 4	2.5	0.3	2	0.2	7

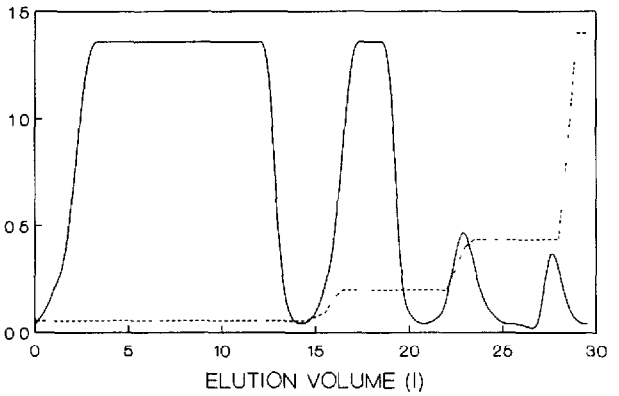

Fig. 1. Purification of C 1 INH on CM-Sepharose Fast Flow. Solid line, absorbance at 280 nm; dashed line, conductivity (S).

volume. Thus the CM-Sepharose effluent required concentration but only minimal diafiltration to achieve a formulation suitable for clinical use.

DISCUSSION

The overall process, including the stepwise elution of C 1 INH from CM-Sepharose, results in a 16% recovery of the C 1 INH at a specific activity of 6.75 U/mg of protein. These results are similar to those reported by Wickerhauser et al. [1]. These authors used salt precipitation as the final step in recovery of a high-purity C 1 INH fraction. In our experience the CM-Sepharose step is simpler and has a shorter process time than salt precipitation. Moreover, the chromatographic method is ideally suited to automation and fits more conveniently into an existing, principally chromatographic, production sequence.

The final product was sterile, free from abnormal toxicity and apyrogenic, and could be heat-treated with minimal loss of activity. The process is suitable for routine large-scale production of a C 1 INH concentrate for clinical use. The in vivo recovery and tolerance of this product will be reported in a further publication.

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