PURIFICATION OF HUMAN α_1-ANTITRYPSIN BY AFFINITY CHROMATOGRAPHY ON SEPHAROSE BOUND CONCANAVALIN A*

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1. Introduction

Quantitatively α_1-antitrypsin (AT) is the most important protease inhibitor in the human serum [1]. It can inhibit several proteases, but its most important physiological role is the inhibition of plasmin. Increased incidence of pulmonary emphysema [2] and certain types of liver cirrhosis [3] were reported in cases of inherited AT deficiency. Until recently the major difficulty in the purification of AT was to separate it from albumin. In the past, several workers have separated AT from albumin at around pH 4.9 but the former readily becomes denatured under this condition [4–7]. Recently in this laboratory AT was separated from albumin by precipitation of the latter with histone, followed by chromatography on DEAE-cellulose [8]. As this technique was rather involved, it was desirable to find a simpler one. Concanavalin A forms insoluble complexes with polysaccharides [9, 10] and some glycoproteins [11]. This paper reports the application of concanavalin A to the separation of AT from albumin, based on the finding that Sepharose-bound concanavalin A, when tested with serum, did bind α-globulins along with other glycoproteins, but did not bind albumin [12].

2. Materials and methods

Con A-Sepharose was purchased from Pharmacia Fine Chemicals. Thrice crystallized bovine α-chymotrypsin (EC 3.4.4.5) (lot #6144-5) and twice crystallized bovine trypsin (EC 3.4.4.4) (lot #9 B) were obtained from Worthington Biochemical Corporation. The commercial trypsin was further purified in this laboratory by chromatography on a carboxymethyl cellulose column. Antisera for immunoelectrophoresis were purchased from the Hoechst Company.

Protein concentrations were measured by the method of Lowry [13] and trypsin and chymotrypsin were assayed with *p*-tosyl-l-arginine methylester and *n*-benzoyl-l-tyrosine ethylester, respectively [14]. Analytical electrophoresis on cellulose acetate membrane was done in a Beckman Microzone instrument in diethyl barbiturate buffer, pH 8.6 for 30 min and the membrane was stained with Ponceau S.

Immunoelectrophoresis was also carried out in the

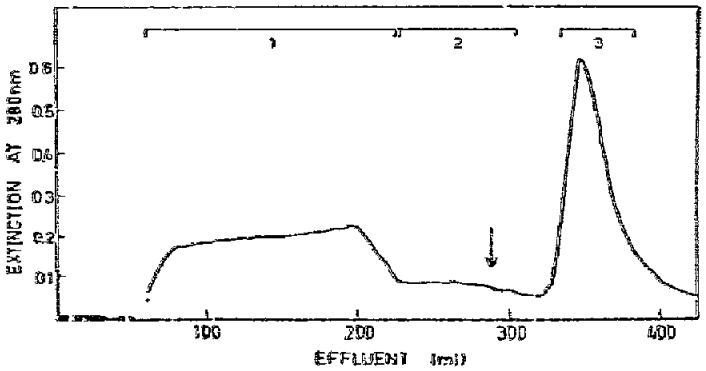

Fig. 1. Chromatography of partially purified AT on Con A-Sepharose. AT (132 ml) was applied to the column at the starting point on the elution graph. After the exhaustion of the sample the column was washed with 10 mM acetate buffer, pH 5.8, containing 0.9% NaCl and at the point indicated by the arrow, elution was started with 50 mM α-methyl D (+)-glucoside dissolved in the same medium.

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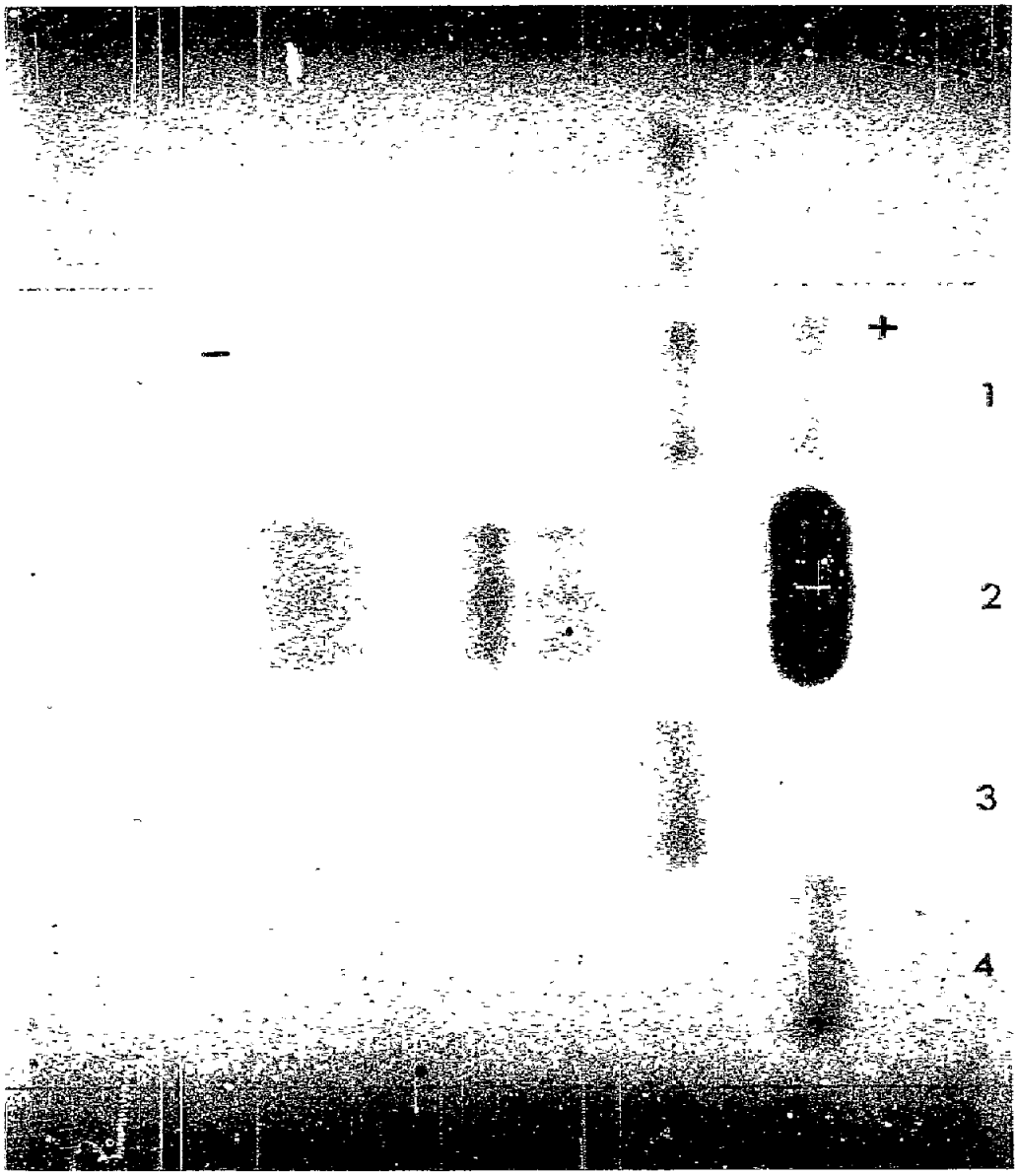

Fig. 2. Electropherogram of fractions containing AT on cellulose acetate membrane. 1, control; 2, human serum; 3, pool 3 (fig. 1); 4, pool 1 (fig. 1).

above buffer at 9.5 V/cm, in 1.25% SeaKem agarose. The running time was 1 hr.

Preliminary purification of AT took place in two steps [8]. Pooled and dialyzed human serum (750 ml) was chromatographed on a Cellex D column (4 × 26 cm) and the inhibitor-containing fractions were dialyzed and freeze-dried. In the second stage, 200 mg portions of the freeze-dried material were subjected to electrophoresis in 7.5% polyacrylamide gel (15.8 cm^2 × 11 cm) in a Buchler "Polyprep" apparatus.

After dialysis, the AT-containing fraction (132 ml, 0.73 mg protein/ml) was chromatographed in 10 mM acetate buffer, pH 5.8, containing 0.9% NaCl on a Con A-Sepharose column (1 × 18 cm) at 4°.

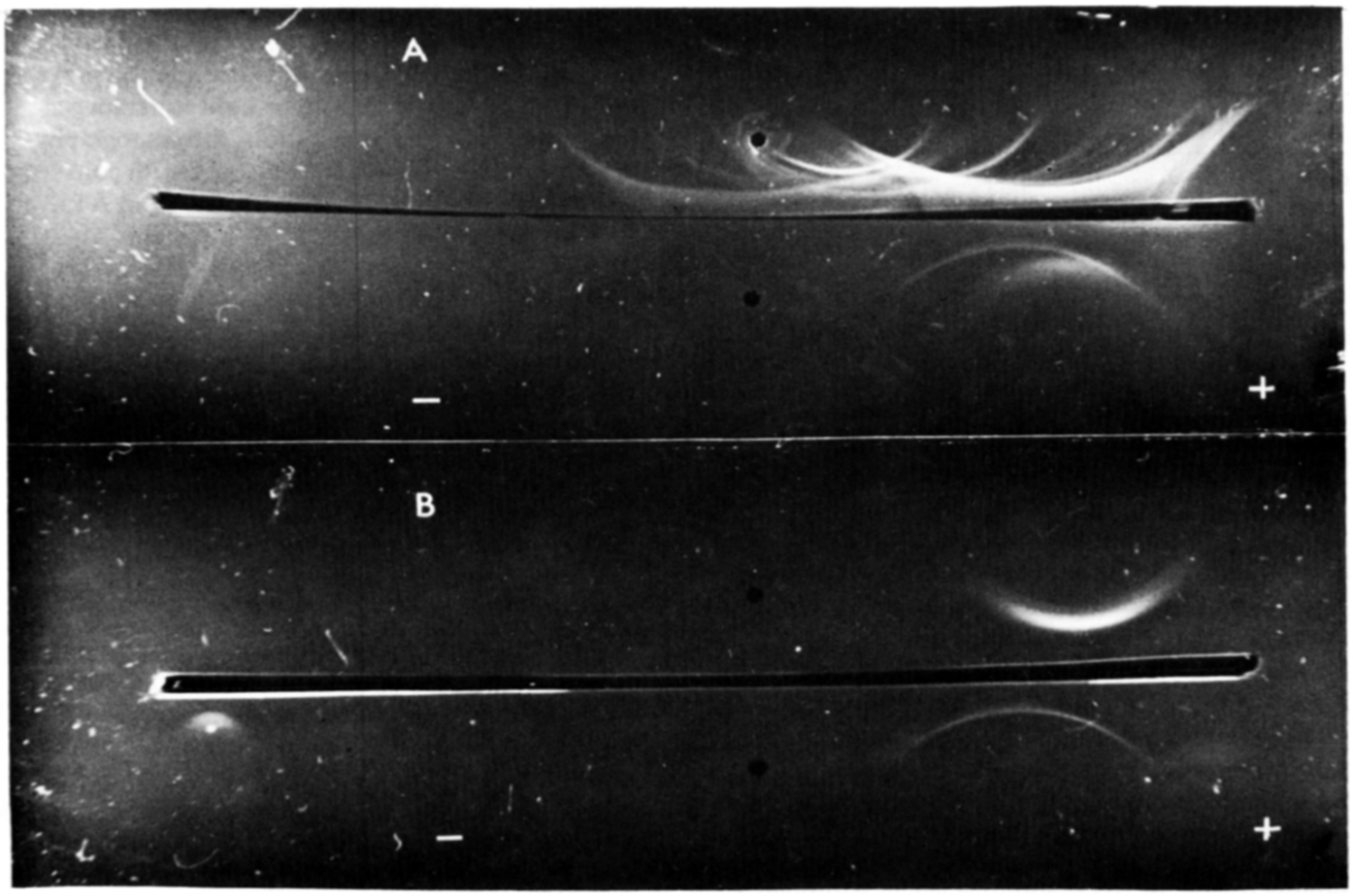

Fig. 3. Immunoelectrophoregram of fractions containing AT. A, top well: 1.0 µl human serum, bottom well: control. B, top well: pool 1 (fig. 1); bottom well: pool 3 (fig. 1). Sample load: approx. 1.0 µg protein, except for serum. Middle trough (A and B): 30 µl anti-human serum (rabbit).

3. Results and discussion

Affinity chromatography of partially purified human AT on a Con A-Sepharose column is shown in fig. 1. The protein in pool 1 emerged only after a fluid volume of 60 ml pased the column. As the total bed volume was only 57 ml, pool 1 protein was retarded to some extent by Con A-Sepharose, but eventually was recovered without applying specific eluting agent. The protein corresponding to pool 3 emerged from the column only after specific displacement with α-methyl D(+)-glucoside.

Electrophoretic examination of aliquots revealed that the partially purified AT (the control) consisted of albumin and α_1-globulin, pool 1 contained albumin only and pool 3 contained α_1-globulin only (fig. 2).

The complete separation of AT from albumin was confirmed by immunoelectrophoresis (fig. 3). The position and shape of the immunoprecipitation line of the pool 3 protein corresponded to AT, and no precipitation line indicative of albumin was visible in the same aliquot.

By immunoelectrophoretic examination against *specific* antisera, no albumin, α_1-acid glycoprotein or α_1-lipoprotein were found in pool 3 protein (not shown).

According to the figures in table 1, 45% of the inhibitor was recovered in pool 3 with a specific trypsin inhibitory potency of 0.40. The specific trypsin inhi-

Table 1
Chromatography of partially purified α_1-antitrypsin on con A-Sepharose: yield and specific inhibition.

	Control	Pool 1	Pool 3
Protein			
(mg/fraction)	96.0	29.1	29.0
Specific inhibition*			
Trypsin	0.27	<0.01	0.40
α-Chymotrypsin	0.24	<0.01	0.41
Yield			
Protein (%)	100	30.4	30.2
Inhibitory capacity (%)**	100	0	45.0

* mg proteinase inhibited per mg inhibitor *protein*.
** Calculated from the trypsin inhibition values of the fractions.

bitory potency of the serum used in this purification was 0.018, thus the overall in the specific inhibition of the product was over 22-fold.

Assuming that 1 mole of protease is bound per mole of inhibitor and taking the molecular weight of AT as 54,000 [15, 16], after making correction for a carbohydrate content of 12%, 1.0 mg AT protein should inhibit 0.52 mg chymotrypsin and 0.42 mg trypsin. In the case of trypsin, the experimental figure (table 1) agrees within a narrow limit with the theoretical figure, but in the case of chymotrypsin, it amounts to 80% of the theoretical value. Although a 20% impurity in the trypsin used could explain this difference, the possibility that AT may have a somewhat higher inhibitory capacity to trypsin than to chymotrypsin, should not be overlooked.

The content of pool 2 was not analyzed in this experiment but corresponding fractions in prior experiments were electrophoretically indistinguishable from the content of pool 3. However, the specific inhibitory potency of those fractions was only about 40% of that corresponding to pool 3 protein. We tentatively assume that the pool 2 proteins corresponded to partially denatured AT.

In a recent work, Myerowitz et al., separated human and mouse AT from albumin by affinity chromatography on a column of Separose-bound anti-albumin globulin [17, 18]. The specific trypsin inhibitory potency of the mouse AT after the last stage of purification was 59% of the theoretical value. The present procedure for the separation of AT from albumin is no less efficient than that of Myerowitz et al., and it eliminates the need to prepare anti-albumin globulin and to couple it with activated Sepharose. An additional consideration is that while the procedure of Myerowitz et al., can be used to remove albumin only from the preparation, chromatography on Con A-Sepharose will separate all serum components that do not react with Con A, from AT.

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