THE PURIFICATION OF HUMAN SERUM α_1-ANTITRYPSIN BY AFFINITY CHROMATOGRAPHY ON CONCANAVALIN A

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SUMMARY

α_1-Antitrypsin (α_1-AT) has been isolated from human serum by a two-step procedure which involves chromatography on DEAE-Sephadex followed by affinity chromatography on insolubilized concanavalin A. This protein appeared to be homogeneous when examined by electrophoresis on cellulose acetate and double immunodiffusion; minor contaminants, however, were detected by gel electrophoresis and immunoelectrophoresis. This procedure is readily adaptable to the large-scale purification of α_1-AT and should facilitate further studies on the physicochemical and biological properties of α_1-AT and its genetic variants.

α_1-AT of human serum is a glycoprotein which has received considerable attention of late because an inherited deficiency of this blood component has been associated with severe pulmonary emphysema (1), iodiopathic distress syndrome in infants (2,3), and liver cirrhosis in children (4) and adults (5). Our knowledge concerning the properties of this protein, however, has been limited by the difficulty involved in its isolation due to its instability below pH 5 and its close association with serum albumin (6-8). Myerowitz et al. (9) have recently reported the purification of α_1-AT from human serum by a procedure which includes as one of its steps the removal of serum albumin with Sepharose-bound antibody to this protein. Their procedure, however, does not lend itself to the isolation of α_1-AT in the quantity needed for the detailed physicochemical characterization of this protein; furthermore the overall recovery of activity was only 4%.

By taking advantage of the known affinity of concanavalin A (con A) for glycoproteins (10), we have been able to obtain a highly purified, active

preparation of α_1-AT by a relatively simple procedure which is readily adaptable to the large-scale production of this protein.

MATERIALS AND METHODS

A convenient starting material is that fraction of pooled human serum (obtained from War Memorial Blood Bank, Minneapolis, Minn.) which is precipitated between 50% and 70% saturation with $(NH_4)_2SO_4$. Approximately 5 g of dialyzed, lyophilized protein was obtained per 100 ml serum. Although only 50% to 60% of the total trypsin inhibitor capacity of the serum is recovered in this fraction, this initial fractionation does serve to eliminate much of the γ-globulins and α_2-macroglobulin which could prove troublesome in subsequent steps of the purification.

Con A was prepared from jack bean meal (Sigma) according to Agrawal and Goldstein (11) and bound to Sepharose-4B which had been activated with cyanogen bromide (12). Sepharose-4B and DEAE-Sephadex A-50 were purchased from Pharmacia Fine Chemicals, Inc., and methyl-α-D-glucopyranoside from Pfanstiehl Laboratories, Inc. Horse antiserum to human serum and monospecific goat antiserum to human α_1-AT were products of Kallestad Laboratories.

Antitryptic activity was determined against 2 x crystallized trypsin (Worthington) using benzoyl-DL-arginine-p-nitroanilide as substrate (13). Inhibitor activity was expressed as μg trypsin inhibited referred to a standard curve employing various levels of trypsin. Specific activity was estimated by dividing inhibitor activity by the absorbance of the test solution at 280 nm. The concentration of α_1-AT was determined by radial immunodiffusion (14) using a commerical kit designed for this purpose (Miles Laboratories, Inc.). Disc electrophoresis was performed on polyacrylamide gel (15) and double diffusion by the Ouchterlony plate technique (16). The Helena ZipZone system was employed for electrophoresis and immunoelectrophoresis on cellulose acetate following instructions provided by the manufacturer (17).

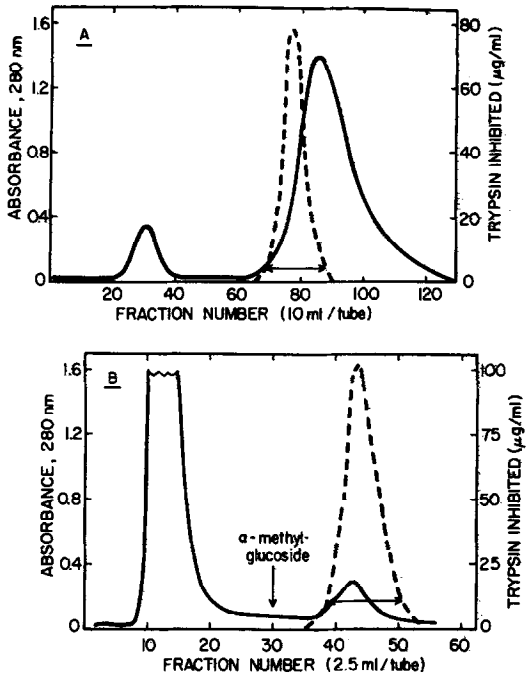


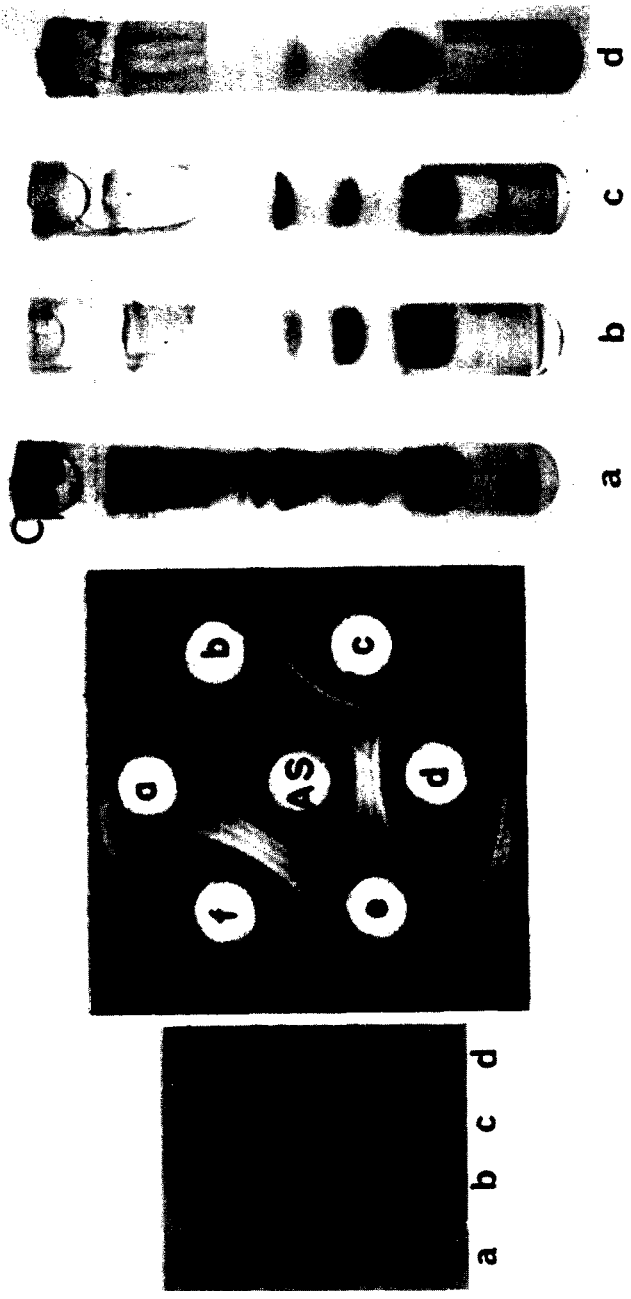
Fig. 1. Purification of α_1-AT from human serum.

A. Chromatography of $(NH_4)_2SO_4$ fraction of human serum. Column (2.5 x 30 cm) was loaded with 0.5 g protein and eluted with 0.05 M phosphate buffer, pH 7.6, with a linear gradient to same buffer containing 0.5 M NaCl (total volume of gradient, 2 l). Fractions (10 ml) were collected at a flow rate of 60 ml/hr; temperature, 4°. Absorbance at 280 nm is shown by solid line and activity by dashed line. Tubes denoted by double-headed arrow were pooled.

B. Chromatography on Sepharose-bound con A. Active fraction from DEAE-Sephadex column was applied directly to column (1.5 x 10 cm) of Sepharose-bound con A and eluted with 0.05 M phosphate buffer, pH 7.6. At point denoted by vertical arrow 0.1 M solution of methyl-α-D-glucopyranoside in same buffer was introduced. Fractions (2.5 ml) were collected at a flow rate of 15 ml/hr; temperature 4°. Tubes corresponding to double-headed arrow were pooled.

RESULTS

In Fig. 1A is shown the pattern obtained when the $(NH_4)_2SO_4$ fraction of human serum was chromatographed on DEAE-Sephadex. The quantity of protein placed on the column was 0.5 g which is equivalent to 10 ml of serum. Approximately 60%-70% of the inhibitor activity originally present in this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fraction could be recovered in that portion of the eluate which corresponded to the leading edge of the main protein peak. Examination of this fraction by electrophoresis on cellulose acetate or polyacrylamide gel (Fig. 2A and C) revealed gross "contamination" with serum albumin.

When the active fraction from the DEAE-Sephadex column was passed through a column of Sepharose-bound con A, the protein which emerged in the hold-up volume consisted largely of serum albumin (Fig. 1B). Subsequent elution with methyl-α-D-glucopyranoside, however, displaced a small amount of protein which accounted for 75% to 80% of the activity which had been applied to the column. This fraction was dialyzed against water to remove excess salts and sugar and was finally lyophilized.

Table I summarizes the data relating to the recovery of activity and to the specific activity obtained during the course of the purification of α_1-AT. About 50% of the activity present in the $(NH_4)_2SO_4$ fraction could be recovered in the final purified fraction with more than a 15-fold increase in specific activity. Since only half of the total trypsin inhibitor capacity of the original serum is present in the $(NH_4)_2SO_4$ fraction, the overall recovery with respect to serum is close to 25%. The yield of purified α_1-AT as represented by the material that could be isolated from the con A column after dialysis and lyophilization was about 8 mg. The α_1-AT content

Fig. 2. Evaluation of homogeneity of fractions containing α_1-AT.

A. Electrophoresis on cellulose acetate. a, active fraction from Sepharose-con A; b, active fraction from DEAE-Sephadex; c, $(NH_4)_2SO_4$ fraction of human serum; d, human serum.

B. Double diffusion. a,b, and e, active fraction from Sepharose-con A; c, active fraction from DEAE-Sephadex; d, $(NH_4)_2SO_4$ fraction from human serum; f, human serum; AS, anti-human serum.

C. Disc gel electrophoresis. a, human serum; b, $(NH_4)_2SO_4$ fraction from human serum; c, active fraction from DEAE-Sephadex; d, active fraction from Sepharose-con A.

D. Immunoelectrophoresis (drawn reproduction). a, human serum; b, active fraction from Sepharose-con A; AS, trough containing anti-human serum.

TABLE I

Recovery of protein and inhibitor activity during the purification of α_1-AT from human serum

Step	Total activity[a]	Specific activity[b]	Activity recovered %	Purification factor
$(NH_4)_2SO_4$ fraction[c]	5830	17	100	1.0
DEAE-Sephadex[d]	3700	67	64	3.9
Sepharose-con A[e]	2970	282	51	16.6

[a] µg trypsin inhibited

[b] µg trypsin inhibited/absorbance at 280 nm

[c] Contains 0.5 g protein equivalent to 10 ml serum

[d] Pooled active fraction shown in Fig. 1A

[e] Pooled active fraction shown in Fig. 1B

of the serum used in this study, as measured by radial immunodiffusion against antiserum to human α_1-AT (14), was 3.1 mg/ml, which is within the normal range reported for healthy individuals (18). Thus the overall recovery of α_1-AT on a weight basis is likewise about 25%.

The results of the several techniques that were used to evaluate the homogeneity of the purified α_1-AT are shown in Fig. 2. Although relatively insensitive to the discrimination of minor components, electrophoresis on cellulose acetate (Fig. 2A) did reveal that chromatography on Sepharose-bound con A was very effective in removing serum albumin. The preparation resulting from this treatment exhibited only one readily discernible precipitin line in the double diffusion test against anti-human serum (Fig. 2B); this line completely fused with the corresponding lines produced by serum itself as well as by the other partially purified fractions. Disc gel elec-

trophoresis (Fig. 2C) showed that the con A purified material contained one major component which lay just behind the position where serum albumin would normally have appeared. A trace of a second component could also be noted which was not identified but which was located in the same relative position as that reported for transferrin (19). Immunoelectrophoresis of the con A purified fraction against anti-human serum (Fig. 2D) likewise revealed one major precipitin arc whose identity as α_1-AT was confirmed by immunoelectrophoresis against monospecific antiserum to human α_1-AT (not shown). In addition to α_1-AT two other faint precipitin lines were apparent. If these are assumed to be glycoproteins, their position in the immunoelectrophoretic pattern would suggest their identity to be α_1-acid glycoprotein and tryptophan-poor α_1-glycoprotein (18). For some unexplained reason, no precipitin line which could be attributed to transferrin was noted in the immunoelectrophoretic pattern of purified α_1-AT.

DISCUSSION

By a relatively simple procedure involving chromatography on DEAE-Sephadex followed by affinity chromatography on insolubilized con A it has been possible to isolate α_1-AT from human serum in a highly purified and active state. Although the equivalent of only 10 ml of serum was processed by the procedure described here, no operational difficulties have since been encountered in scaling up the various steps in this procedure by a factor of 10 by employing proportionately larger columns and volumes of buffer. The yield of α_1-AT is proportionately greater with essentially no sacrifice in the purity of the isolated inhibitor.

Although the α_1-AT isolated in this study was not completely devoid of contaminants, the quantity of such impurities must be small indeed. The material isolated by this procedure could probably be used directly for many studies on the physicochemical and biological properties of α_1-AT where absolute purity is not essential. It can also serve as a convenient point of departure for the further purification of α_1-AT, which would of course

be highly desirable for the production of monospecific antisera. Another important aspect of this problem which should now be facilitated is the characterization of the several genetic variants of α_1-AT which are known to be present in the blood of normal and α_1-AT deficient individuals (20,21). Studies along these various lines are currently in progress in this laboratory.

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