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Purification and Characterization of Canine α-1-Antiproteinase†

William R. Abrams,* Philip Kimbel, and George Weinbaum

ABSTRACT: The principal canine plasma protease inhibitor, α-1-antiproteinase, has been purified 90-fold with a 25% yield to apparent homogeneity. The purification scheme includes anion-exchange chromatography, to separate away the bulk of the serum albumin; affinity chromatography by insolubilized concanavalin A, to remove most of the other serum proteins as well as traces of albumin; and, finally, sizing on Sephacryl-S-200. Unique to this purification scheme is the batch use of insolubilized hemoglobin–Sepharose beads to remove the ubiquitous contaminant haptoglobin. The purified material has an apparent molecular weight of 58 000, 11.2% carbohydrate, and an $E_{280nm}^{1\%} = 5.82$, and can be separated by isoelectric focusing into at least two distinct forms with p*I* values of 4.40 and 4.52. In addition, canine α-1-antiproteinase is immunologically distinct from human α-1-antiproteinase.

Over the past several years this laboratory has been working with a homologous animal model system for the study of the etiology of experimental canine emphysema (Marco et al., 1971; Mass et al., 1972; Weinbaum et al., 1974; Janoff et al., 1977). During the course of these studies it became necessary to purify canine α-1-antiproteinase (AP)[1] to serve as an antigenic and biochemical standard for differentiation of the various protease inhibitors found in the dog lung (Weinbaum et al., 1976).

Biochemically, AP is unusual because it is approximately twice the size of the serine proteases (i.e., trypsin and elastase) that it inhibits. It is difficult to purify in large quantities free from serum albumin, transferrin, and haptoglobin, although it is found in the normal dog at an abundant level of 150–200 mg/100 mL of serum (Kueppers, 1977, personal communication). Its presence is ubiquitous throughout the body as a normal component of serum as well as being associated with cell types such as platelets (Nachman and Harpel, 1976; Nalli et al., 1977) and macrophages (Cohen, 1973). In general terms, it is believed to be a broad spectrum inhibitor which modulates protease activities in conjunction with α-2-macroglobulin (Ohlsson, 1971).

In recent years, several different techniques have been applied to the purification of human AP, i.e., ion-exchange chromatography (Crawford, 1973; Chan et al., 1973; Berninger and Mathis, 1976; Hercz and Barton, 1977; Myerowitz et al., 1972a), affinity and gel-filtration chromatography (Liener et al., 1973; Murthy and Hercz, 1973; Travis et al., 1976), or thiol interchange chromatography (Laurell et al., 1975, 1977). The work reported in this paper draws in part from all these techniques. This paper presents a simple, rapid, large-scale purification of canine AP to homogeneity with high yield. In addition, data are presented which suggest that canine AP consists of at least two isoinhibitors.

Experimental Procedure

Materials

Chemicals and enzymes were obtained as follows: hemoglobin, benzamidine, polybrene (1,5-dimethyl-1,5-diazauradecamethylene polymethobromide), 2-mercaptoethanol, 1-*O*-methyl α-D-glucopyranoside, transferrin (human), aldolase, and ovalbumin were from Sigma Chemical Co.; con-

† From the Pulmonary Disease Section, Department of Medicine, Albert Einstein Medical Center, Philadelphia, Pennsylvania 19141. *Received February 13, 1978.* This work was supported by National Institutes of Health Grants HL13787 and PO1HL20994 and Grant 901 from the Council for Tobacco Research, U.S.A., Inc.

[1] Abbreviations used: $NaDodSO_4$, sodium dodecyl sulfate; Boc-Ala-ONp, *tert*-butyloxycarbonyl-L-alanine *p*-nitrophenyl ester; BAPA, *N*-benzoyl-DL-arginine *p*-nitroanilide; AP, α-1-antiproteinase; EDTA, (ethylenedinitrilo)tetraacetic acid.

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canavalin A–Sepharose (lot BL5958), QAE-A-50 Sephadex, Sephacryl S-200, and Sepharose 4B were from Pharmacia Fine Chemicals; porcine pancreatic elastase (90 IU/mg) was from Worthington Biochemicals; Boc-Ala-ONp was from Pierce Chemical Co.; SAPNA was from the Protein Research Foundation, Osaka, Japan; enzyme-grade ammonium sulfate and two times crystallized bovine serum albumin were from Schwarz/Mann; *N,N'*-methylenebisacrylamide, acrylamide, 1-methyl-2-pyrrolidone, and Coomassie brilliant blue R-250 were from Eastman Kodak Co.; $NaDodSO_4$, recrystallized from ethanol before use, was from Matheson, Coleman, and Bell; PM10 Diaflo ultrafiltration membranes were from Amicon Corp.; PTGC ultrafiltration filters were from Millipore Corp.; BAPA and trypsin, two times crystallized, were from ICN Pharmaceuticals; Ampholines were from LKB Instrument Co., Inc.; goat anti-dog whole serum was from Cappell Laboratories; and rabbit anti-human AP was from Behring Diagnostics.

All other reagents were of the highest commercial grade available and used without further purification. Solutions were made with deionized water of maximum conductivity of 1 to 2 μmho.

Dialysis tubing was purchased from Arthur H. Thomas Co., boiled twice in 0.1% EDTA, and stored at 4 °C.

Methods

Purification. All purification procedures were performed at 4 °C unless indicated otherwise.

Ammonium Sulfate Precipitate (Step 1). To 250 mL of acid-citrated dextrose-treated dog plasma are added 30 mg of polybrene to prevent activation of complement protease activities, 0.3 mmol of benzamidine, 0.6 mmol of 2-mercaptoethanol and 50 mL of 0.05 M phosphate buffer containing 0.1 M NaCl, pH 7.5. This solution is made 50% with saturated ammonium sulfate in 0.01 M phosphate buffer (pH 7.5), stirred for 1 h, and then centrifuged at 17 369*g* for 15 min. The supernatant is made to 80% saturation with solid ammonium sulfate, allowed to stir from a minimum of 2 h to a maximum of 16 h, and then centrifuged as above. The pellet was dissolved in a minimum volume, usually 25 mL of buffer A [0.005 M phosphate, 0.1 M NaCl, 0.001 M 2-mercaptoethanol, 0.001 M benzamidine (pH 6.5), conductivity 7.5 mmho], and dialyzed against several changes of a 40- to 50-fold excess of buffer A.

Ion-Exchange Chromatography (Step 2). A column (3 × 30 cm) of QAE A-50 Sephadex, equilibrated with buffer A, was charged with 2.5–3.5 g total protein from step 1 in a 50–100 mL volume at a flow rate of 50–60 mL/h, followed by washing with buffer A until the 280-nm absorbance falls below 0.1. The eluant is changed to buffer B (buffer A containing 0.16 M NaCl final concentration, pH 6.5, conductivity 11.5 mmho). The active fractions were pooled and concentrated by pressure dialysis to an approximate volume of 25 mL.

Affinity Adsorption (Step 3). To remove haptoglobin and nonspecific proteases, a 10–15-mL packed volume of hemoglobin–Sepharose 4B beads was mixed with 25 mL of protein from step 2 and an additional 25 mL of buffer C (0.05 M sodium acetate, 0.25 M NaCl, 1 mM 2-mercaptoethanol, pH 6.0). This slurry was agitated for 1 h, and the supernatant batch was separated from the beads on a coarse scintered glass filter and dialyzed against buffer C.

Affinity Chromatography (Step 4). To a column (1.9 × 9.0 cm) of concanavalin A–Sepharose equilibrated with buffer C was applied the protein filtrate from step 3 at a flow rate of 10–20 mL/h. When the absorbance at 280 nm fell below 0.2 unit, the column was eluted with buffer C containing 0.1 M 1-*O*-methyl α-D-glucopyranoside. The inhibitor-containing peaks were pooled, dialyzed vs. buffer D (0.01 M phosphate, 0.25 M NaCl, 0.001 M 2-mercaptoethanol, pH 7.5), and concentrated to a volume of 3–5 mL. The column is regenerated by washing with 2 column volumes of 0.1 M sodium acetate (pH 6.0) containing 1 mM each of $CaCl_2$, $MgCl_2$, $MnCl_2$, and 1.0 M NaCl, followed by a 5 column volume wash of buffer C.

Gel Filtration (Step 5). The protein from step 4 is layered onto a column (2.6 × 86 cm) of Sephacryl-S200 and eluted with buffer D at a flow rate of 12 mL/h controlled by a peristaltic pump. Active fractions were pooled and concentrated by pressure filtration and stored frozen at −20 °C.

Pertinent data on the various steps are summarized in Table I.

Antiserum. Antisera were produced in rabbits against a partially purified preparation of a dog AP (from step 3) by the method of Goudie et al. (1966).

Electrophoresis. Slab-gel polyacrylamide electrophoresis was carried out with 0.1% sodium dodecyl sulfate according to the method of Laemmli (1970) and Ames (1974) using a 10% T, 0.27% C gel on an apparatus similar to that designed by Studier (1973). Sample preparation was similar to that detailed by Laemmli (1970), except that samples were boiled for 2 min in the sample buffer.

A native pH 8.3 (7% T, 0.18% C) slab gel was based on the method of Davis (1964). Samples were dissolved in 0.5 M sucrose to facilitate layering under the upper electrode buffer. Both native and $NaDodSO_4$ gels were fixed in 50:50:10 MeOH–H_2O–Ac, stained at 37 °C with gentle agitation in the fix solution containing 0.2% Coomassie brilliant blue R-250, destained in fix solution, and swollen in 80:10:10 H_2O–MeOH–Ac. Gels were stored by drying onto photographic blotter paper with a Hoeffer slab drying apparatus.

Amino Acid Analysis. Amino acid analyses were performed on samples hydrolyzed for 24, 48, and 72 h in 6 N HCl at 110 °C on a Jelco JLC-5AH amino acid analyzer. The half-cystine residues were stabilized during the purification by the presence of 2-mercaptoethanol. Loss during hydrolysis of threonine, serine, methionine, and tyrosine was corrected for by extrapolation to zero time. Tryptophan was not determined.

Carbohydrate Content. Samples were exhaustively dialyzed against water prior to digestion. Total carbohydrate was determined according to the method of Park and Johnson (1949), utilizing hydrolysis at 100 °C in 2 N H_2SO_4 for 4 h, followed by neutralization with NaOH. Carbohydrate content was calculated using glucose as the reference compound.

Protein Content. Protein was determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard.

Hemoglobin–Sepharose. Hemoglobin was cross-linked to Sepharose 4B according to the method of Cuatrecasas and Anfinsen (1971), as modified by March et al. (1974). Hemoglobin was used in the approximate ratio of 40 mg/g of cross-linked gel.

Isoelectric Focusing. Isoelectric focusing was performed according to the method of Vesterberg and Svenson (1966) and the LKB Manufacturers Operating Manual in a 110-mL capacity column at 4 °C for 43 h. Carrier ampholines were supported in a 0 to 25% linear sucrose gradient and utilized in the following concentration for each pH range: 0.19%, pH 3–3.5; 1.0%, pH 3.5–5.0; 0.19%, pH 7.0–10.0. The constant voltage applied to the column was increased in steps from 350 to 500 V during the first 8 h of the focusing while keeping the total power below 2 W. One-milliliter samples were collected and tested for inhibitory activity, and the pH was determined

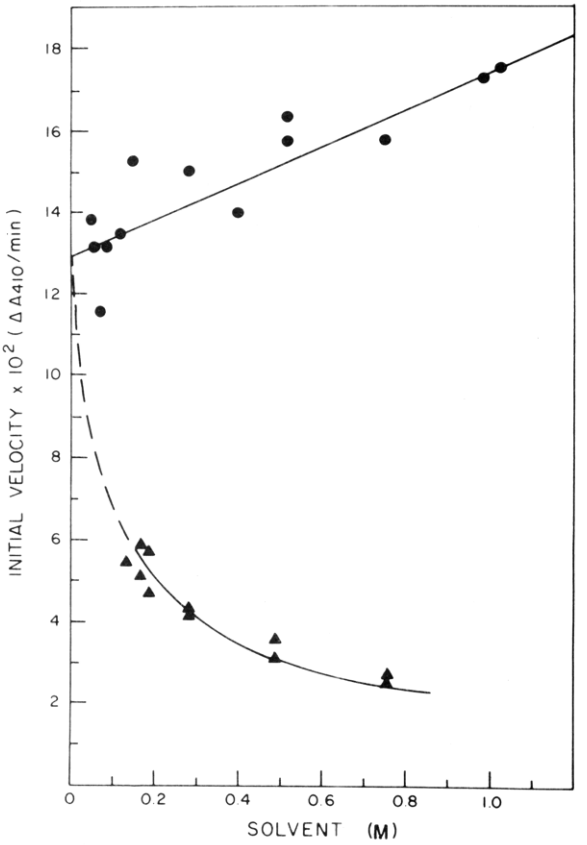

FIGURE 1: The dependence of the initial velocity of hydrolysis of Boc-Ala-ONp on substrate solvent. Hydrolysis by pancreatic elastase at 410 nm and 25 °C is shown as a function of dimethyl sulfoxide (●) and acetonitrile (▲): Elastase = 0.83 μg/mL; Boc-Ala-ONp = 0.66 mM.

with a Radiometer pH M26 meter equipped with a GK2321C electrode equilibrated and calibrated at 4 °C at pH 4.0, 7.1, and 8.6.

Immunoelectrophoresis. Immunoelectrophoresis was performed in a Millipore Four-Cell using a pH 8.6, 0.07 M barbital, 0.05 M boric acid, 0.01 M Na_2HPO_4 buffer for 30 min at 100 V using Millipore Immuno-Agaroslides. After deproteinization, slides were stained with 1% amido black in 5% acetic acid and destained in 5% acetic acid.

Assays. Inhibitory activity of AP was monitored as the residual fraction of elastase activity when compared to the control. One inhibitory unit (IU) represents that amount of inhibitor required to reduce the initial velocity of a 1.0 μg/mL solution of pancreatic elastase to zero. A modification of the elastase assay method of Visser and Blout (1972) was used as follows: the substrate, Boc-Ala-ONp was prepared as a 6 mg/mL solution in dimethyl sulfoxide and subsequently diluted to 0.06 mg/mL in 0.05 M phosphate buffer (pH 6.5) as the substrate solution. The inhibitor-containing sample was incubated for 15 min at 25 °C with 3.1 μg of pancreatic elastase in an initial volume of 0.026–0.05 mL and diluted with 3.0 mL of the substrate solution (to give approximately a 3.1-mL final volume), and the release of *p*-nitrophenol was followed at 410 nm in a Gilford Model 2000 spectrophotometer.

Trypsin inhibition was measured by the method of Erlanger et al. (1961) using BAPA, by following the continuous release of *p*-nitroaniline at 25 °C and pH 8.0. The same preincubation schedule described above for elastase was used.

Results and Discussion

Assays. Initially, acetonitrile was utilized as a solvent to dissolve the substrate, Boc-Ala-ONp, in the elastase assay of

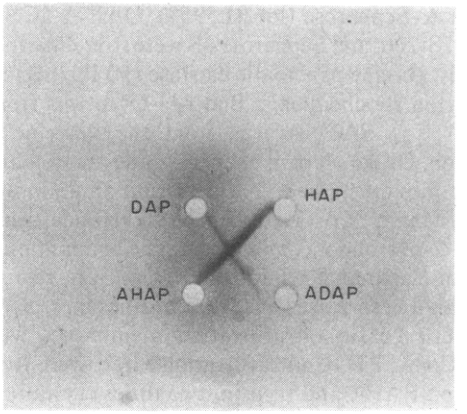

FIGURE 2: Double immunodiffusion test between human AP (HAP), rabbit anti-human AP (AHAP), dog AP (DAP), and rabbit anti-dog AP (ADAP).

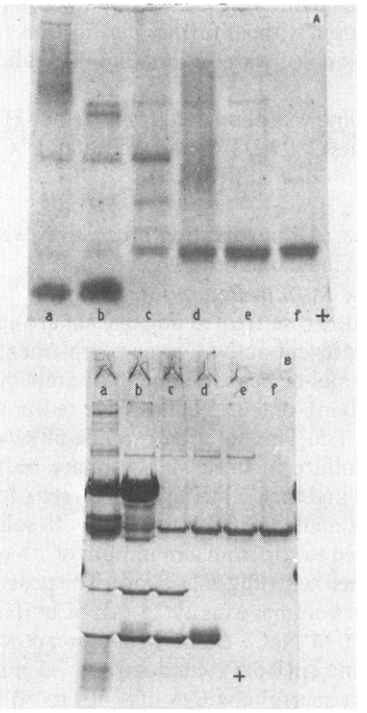

FIGURE 3: (A) Alkaline slab-gel electrophoresis of dog AP samples obtained during the purification: (a, 13.2 μg of dog plasma (starting material); b, 10.5 μg of 50–80% ammonium sulfate fraction (step 1); c, 10 μg from anion-exchange chromatography at pH 6.5 (step 2); d, 10 μg from concanavalin A–Sepharose chromatography (step 4); f, 8.6 μg from Sephacryl-S200 chromatography (step 5). (B) Sodium dodecyl sulfate slab-gel electrophoresis of dog AP samples obtained during the purification. The samples applied to the corresponding sample wells were the same as in A, except for changes in protein content: a, 20 μg; b, 10.5 μg; c, 6 μg; d, 6 μg; e, 5.4 μg; f, 4.5 μg. The gel was calibrated with respect to molecular weight by running the following proteins: human transferrin, bovine serum albumin, ovalbumin, and aldolase monomer.

Visser and Blout (1972). The final concentration of acetonitrile would be 0.19 M, which is sufficient to inhibit 80% of the active elastase based on an apparent K_i of 100 mM (Figure 1). Because the purification necessitated monitoring inhibitory activity, the possibility of an additive inhibitory effect on the apparent K_m or K_i was eliminated by the use of dimethyl sulfoxide. This solvent at a final concentration of 0.14 M in the reaction mixture had no apparent inhibitory effect (Figure 1).

When dog AP was purified to homogeneity by the techniques described below, it was shown that there was no im-

TABLE I: Purification of Dog α-1-Antiproteinase.

procedure	protein (mg)	sp act.[a] (IU/mg of protein)	AP recovery (%)
plasma (start. material)	16 500	4.6	100
50–80% $(NH_4)_2SO_4$ (step 1)	4 270	8.6	49
QAE A-50 Sephadex chromatogr (step 2)	305	89.3	36
Hb–Sepharose batch extract. (step 3)	149	147.3	29
Con A–Sepharose chromatogr (step 4)	77	289.8	29
Sephacryl S-200 chromatogr (step 5)	46	418.0	25

[a] For a 1:1 association between AP and elastase, with molecular weights of 25 900 (Shotton and Harley, 1973) and 58 000 (this work), respectively, the theoretical specific activity for pure AP is 446 IU/mg of inhibitor protein.

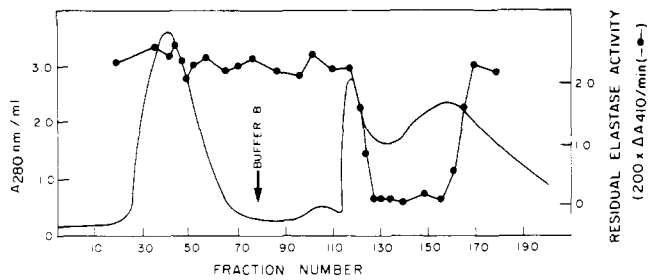

FIGURE 4: QAE-A-50 Sephadex anion-exchange chromatography of AP from step 1 in buffer A. Elution with buffer B was initiated at fraction 79. The solid line is proportional to the absorbance at 280 nm, as measured in a UV flow monitor. Inhibition by AP is indicated as the remaining activity of elastase against Boc-Ala-ONp after a preincubation of 0.05 mL of each column sample (●). Fractions of 3.2 mL were collected, and samples numbered 127 to 164 were pooled and represent step 2.

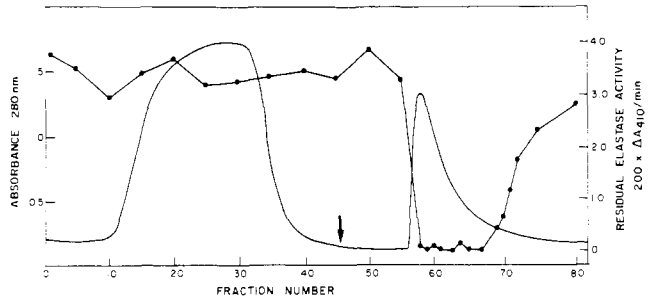

FIGURE 5: Concanavalin A–Sepharose chromatography of AP from step 3 in buffer C. Elution with buffer C containing 0.1 M 1-*O*-methyl α-D-glucopyranoside was initiated at fraction 45. The solid line is proportional to the absorbance at 280 nm, as measured in a UV flow monitor. Inhibition by AP is indicated as the remaining activity of elastase against Boc-Ala-ONp after a preincubation of 0.035 mL of each column sample (●). Fractions of 3.2 mL were collected, and samples numbered 56 to 75 were pooled and represent step 4.

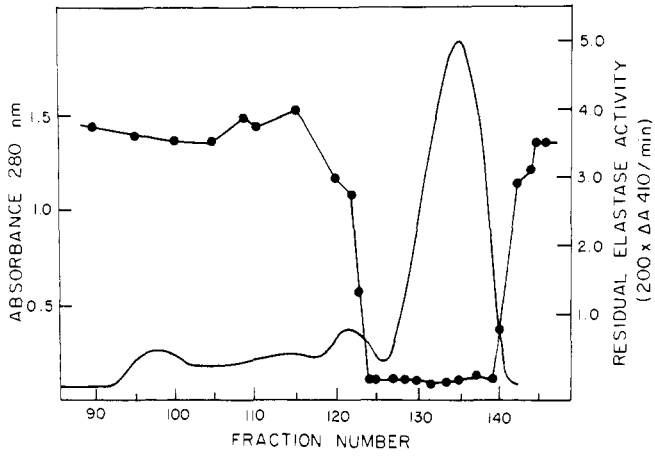

FIGURE 6: Sephacryl S-200 chromatography of AP from step 4 in buffer D. The solid line is proportional to the absorbance at 280 nm as measured in a UV flow monitor. Inhibition by AP is indicated as the remaining activity of elastase against Boc-Ala-ONp after a preincubation of 0.025 mL of each column fraction (●). Fractions of 1.1 mL were collected, and sample numbers 128 to 142 were pooled and represent step 5.

munological cross-reactivity between dog and human AP (Figure 2). This made the immunological monitoring of the purification using commercially available antibodies against human AP impossible. The spectrophotometric determination of AP by inhibition of elastase hydrolysis of Boc-Ala-ONp was quick, convenient, and inexpensive. In addition, Senior et al. (1972) have shown that serum elastase inhibitory capacity closely reflects serum antiproteinase concentration.

Purification. The progress of the purification is demonstrated on analytical slab polyacrylamide gels in Figure 3, and the data are presented in Table I. The samples correspond to appropriate steps in the purification for both native and $NaDodSO_4$ gels, as indicated in the legend of Figure 3.

Treatment of plasma with 1 mM phenylmethanesulfonyl fluoride prior to ammonium sulfate fractionation does not appear to improve the yield of recoverable inhibitor. The second step utilized ion-exchange chromatography and effects a significant purification (Figure 4) with careful control of the eluting buffer's ionic strength. This step separates AP from the bulk of serum albumin. α-1-acid glycoprotein is also removed from the AP preparation in step 2, by an ion-exchange chromatography at pH 6.5 (Travis et al., 1974), as shown by the lack of an approximately 44 000-dalton molecule (Schmid, 1975) in 0.1% $NaDodSO_4$ gels (Figure 3) or a molecule of higher mobility than AP in standard pH 8.5 polyacrylamide gels (Figure 3).

A major remaining contaminant was found to bind hemoglobin, move with the mobility of an α-2 serum protein on immunoelectrophoresis, and have a 40 000-dalton subunit on $NaDodSO_4$ gels (Figure 3). This protein does not separate from AP in the concanavalin A–Sepharose procedure. All the above properties are consistent with its being haptoglobin. Therefore, advantage was taken of its affinity for hemoglobin in order to remove it prior to the affinity chromatography step. Hemoglobin cross-linked to Sepharose 4B was used in a batch procedure to remove this protein, effecting an almost twofold purification with good recovery. The AP solution develops a brownish cast which has the appearance of dilute hemoglobin and is removed in subsequent operations. This color is most likely due to the action of nonspecific proteases that are bound to the hemoglobin matrix (Chua and Bushuk, 1969; Smith and Turk, 1974) together with haptoglobin.

The chromatography of AP on concanavalin A–Sepharose is shown in Figure 5. The presence of Ca^{2+}, Mg^{2+}, and Mn^{2+} was not required for binding of AP to the column, but they were incorporated into a column-recycling procedure to ensure the stability of the lectin and eluted before application of the solution from step 3 with buffer C.

In this procedure, traces of albumin as well as another unidentified protein with a subunit of approximately 32 400 daltons are removed (Figure 3). It was observed in this work

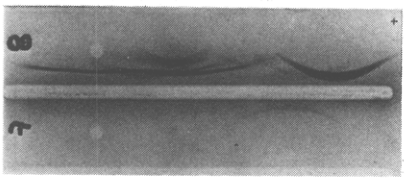

FIGURE 7: Immunoelectrophoresis of dog AP (from step 5) (lower well) and dog whole plasma (upper well). Goat anti-dog whole serum was placed in the trough after electrophoresis and immunoprecipitation was allowed to continue for 24 h.

TABLE II: Comparison of Inhibition by the AP Isomers against Elastase and Trypsin.

isoelect focus. fract	sp act.[a] (IU/mg of protein) elastase (IU_E)	trypsin (IU_T)	(IU_E/IU_T)
55	114	143	0.8
57	178		
62	164	154	1.06

[a] Protein content was determined by the absorption at 280 nm and converted to mg of AP using the extinction: $E_{280nm}^{1\%} = 5.82$.

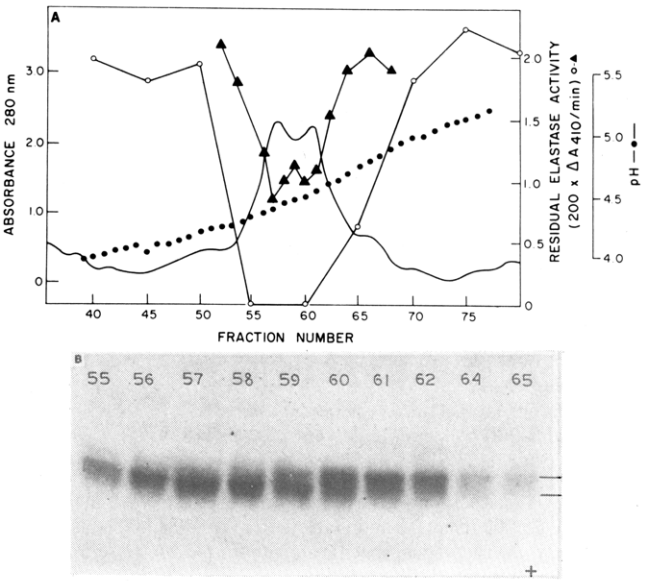

FIGURE 8: (A) Separation of dog AP by electrofocusing. The solid line shows the absorption in the eluate at 280 nm. Inhibition by AP is monitored as the remaining activity of elastase against Boc-Ala-ONp after a preincubation of 0.04 mL (○) or 0.005 mL (▲) of each column fraction tested. (B) Alkaline slab-gel electrophoresis of fractions obtained by electrofocusing AP. The number above each sample position corresponds to the fraction number (Figure 8A). A constant volume of 0.007 mL was used from each fraction.

that a trace amount of phosphate, which readily forms calcium phosphate on exposure to the calcium ions of the concanavalin A–Sepharose matrix, enhances AP binding ability.

The gel-filtration step (Figure 6) effectively separates the remaining contaminant from AP. This contaminant has been characterized only by the relative behavior on $NaDodSO_4$–polyacrylamide gels (Figure 3), which shows that a band at 82 000 daltons was removed.

Immunoelectrophoresis of AP showed a single precipitin arc using goat anti-dog whole serum (Figure 7). However, alkaline native gels show a "doublet", which is attributed to an isomeric form as shown more clearly by isoelectric focusing (Figure 8A). The p*I* values for the two peaks of inhibitory activity are 4.40 and 4.52, respectively. Figure 8B shows the separation of the "doublet" on an alkaline polyacrylamide gel. Both peaks inhibit trypsin and elastase with similar efficiency (Table II) and have electrophoretic mobilities of α-1-globulins. Multiple forms of AP have been detected in rat, mouse, and man using alkaline polyacrylamide gels (Rosenberg et al., 1976; Myerowitz et al., 1972a,b).

The temperature stability of AP was monitored as a function of time by its ability to inhibit porcine pancreatic elastase as measured with Boc-Ala-ONp. When heated in 0.01 M phosphate, pH 7.5, containing 0.1 M NaCl, AP has an inhibitory activity half-life of 3.7 h at 60 °C, while there appears to be no loss of activity at 40 °C over a 4-h period. Samples were stored at −20 °C without loss of activity for periods of up to 6 months; however, repeated freezing and thawing of the same sample resulted in a 10% loss of inhibitory capacity per month. In contrast to the above, it has been reported that human α-1-antitrypsin in serum is destroyed in 10 min at 56 °C (Steinbuch, 1976).

The total carbohydrate content for dog AP was found to be

TABLE III: Amino Acid Analysis.

AA	residues/1000, dog	residues/molecule dog[a]	monkey[b]	man[c]	man[d]
Asp	102.1	41	42	44.82	42.5
Thr	75.7	30	27	28.5	33.2
Ser	62.7	25	22	22.47	27.2
Pro	41.6	17	20	17.27	18.7
Glu	126.9	51	43	50.2	54.0
Gly	58.6	23	24	23.74	22.1
Ala	68.5	27	29	26.61	26.6
Val	57.8	23	27	26.75	23.8
½-Cys	4.0	1.6	2	1.74	1.2
Met	37.5	15	8	7.46	8.6
Ile	44.0	18	17	20.6	18.2
Leu	108.8	44	43	45.03	43.9
Tyr	43.5	17	10	6.89	6.0
Phe	66.2	27	21	28.17	28.3
Lys	79.0	32	35	35.05	36.5
His	27.1	11	16	12.52	12.4
Trp				1.8	2.2
Arg	25.7	10	11	7.46	8.1

[a] Based on a molecular weight of 58 000. [b] Berninger and Mathis (1976). [c] Hercz and Barton (1977). [d] Chan et al. (1973).

11.2% which is comparable to values for monkey of 11.7% (Berninger and Mathis, 1976) and human of 12 and 16.4% (Chan et al., 1973; Chan and Rees, 1976). The amino acid analysis is given in Table III. The results are extrapolated values obtained from hydrolyses for 24, 48, and 72 h.

The extinction at 280 nm for this protein, $E_{280nm}^{1\%} = 5.82$, is similar to that for human $E_{278nm}^{1\%} = 5.3$ (Schonenberger, 1955) and 4.36 (Crawford, 1973). However, there is a lack of immunologic similarity between the human and canine inhibitors (Figure 2). The molecular weight of dog AP was estimated at 58 000 from a linear graph of the log of molecular weights of human transferrin, bovine serum albumin, ovalbumin, and aldolase monomer as a function of their mobility in an alkaline 0.1% $NaDodSO_4$–polyacrylamide gel electrophoresis.

Titration by AP of elastase activity against Boc-Ala-ONp at 25 °C is linear from 0 to 100% inhibition. The association is as predicted for a 1:1 molar interaction.

A method for the purification of dog AP 90-fold with 25% overall yield has been described. The inhibitor was purified to establish a reference component for a completely homologous system investigating the etiology and pathogenesis of emphysema. The characteristics of dog AP are the presence of at least two isoinhibitor forms and its lack of immunological cross-reactivity with human AP. Whether these isoinhibitors correspond to the various p*I* forms in the human is presently unclear. It is now possible to determine if these two antiproteinases interact with known proteases by identical mechanisms.

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