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Arginine esterase
cystic fibrosis

α_2-macroglobulin
proteolytic enzyme

Absence of an α_2-Macroglobulin-Protease Complex in Cystic Fibrosis

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Extract

The present study using immunologic methodology confirms previous observations from this laboratory of an absence of a protease component with arginine esterase activity in plasma of patients with cystic fibrosis. In this study, the pooled plasma from control individuals was activated and partially purified after adsorption on columns of soybean trypsin inhibitor conjugated to Sepharose 4B followed by elution with benzamidine. The fraction was further purified by isoelectrofocusing on polyacrylamide gels. Proteins around the pI range of 5.5 were eluted and utilized to prepare an antiserum. Immunoelectrophoresis of activated plasma samples from control subjects and patients with cystic fibrosis was performed utilizing the antiserum. In controls, four precipitin arcs with residual esterase activity were observed, whereas only three were seen in plasma from patients with cystic fibrosis. Double gel diffusion experiments using specific antisera ruled out the presence of trypsin, chymotrypsin, plasminogen, prothrombin, C1 esterase, α_1-trypsin inhibitor, and inter-α-trypsin inhibitor in the concentrated benzamidine eluate. The antisera to α_2-macroglobulin gave an immunoprecipitate which was readily stained for proteolytic activity. On immunoelectrophoresis, the α_2-macroglobulin precipitin band corresponded to the band absent in plasma of patients with cystic fibrosis. In contrast, the α_2-macroglobulin levels were similar in plasma of control subjects and patients with cystic fibrosis. Using the antiserum to the protein fraction with a pI of 5.5 in cross immunoelectrophoresis, three "rockets" with proteolytic activity could be demonstrated in control plasma. One specific enzyme-active "rocket" was absent in plasma of patients with cystic fibrosis. In a double blind study of 15 control samples and 15 samples from patients with cystic fibrosis, a specific "rocket" was shown to be present in 13 control samples and absent in 14 cystic fibrosis samples. α_2-Macroglobulin was determined by both an immunologic procedure and by its trypsin binding (trypsin protein esterase concentration). The ratio of the immunologic assay to the biologic activity assay was 90 for the normal plasma samples and only 65 for cystic fibrosis samples.

Speculation

The absent α_2-macroglobulin-protease complex in plasma of patients with cystic fibrosis might reflect a molecular defect in either a protease with arginine esterase activity or within the α_2-macroglobulin molecule.

Previous studies have shown that saliva and plasma of patients with cystic fibrosis are deficient in a proteolytic activity with arginine esterase substrate specificity (18–21). Subsequent studies have documented the proteolytic nature of this arginine esterase activity (20). On the basis of these observations, we have speculated that the deficiency of proteolytic activity could explain the presence of several cationic macromolecular "factors" reported to be present in saliva (16) and plasma (4, 5, 25) of patients with cystic fibrosis.

Previous studies of arginine esterase have employed enzymologic, chromatographic, and isoelectrofocusing techniques. In this report, utilizing an immunologic methodology, we demonstrate the absence of an enzyme, α_2-macroglobulin complex in plasma of patients with cystic fibrosis.

EXPERIMENTAL PROCEDURE

MATERIALS

Soybean trypsin inhibitor (STI) type II-S (lot 34C-8180) used for affinity chromatography; trypsin inhibitor type I-S (chromatographically prepared, lot 85C-8055) used for inhibition studies; bovine trypsin type III twice crystallized (lot 21C-0140); α-*N*-benzoyl-DL-arginine-β-naphthylamide-HCl (BANA, lot 44C-0880); α-*N*-benzoyl-L-arginine ethyl ester-HCl (BAEE, lot 94C-0006-2); α-*N*-benzoyl-DL-arginine-*p*-nitroanilide-HCl (BAPNA, lot 124C-0292); *o*-dianisidine, tetrazotized (lot 64C-01012); and *p*-hydroxymercuribenzoate (Na salt, lot 97B-5200) were all obtained from Sigma Chemical Company (St. Louis, Mo.). Benzamidine hydrochloride hydrate (lot 111757) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, Wis.). Agarose was procured from Marine Colloids, Inc. (Rockland, Ma.), and Sepharose 4B was obtained from Pharmacia Fine Chemicals, Inc. (Piscataway, N. J.). All other materials used were reagent grade or the best grade available.

ANTISERA

Specific rabbit antisera to human whole serum, albumin, α_1-antitrypsin, α_2-macroglobulin, inter-α-trypsin inhibitor, plasminogen, prothrombin, and complement component C1 esterase (C1s) were all purchased from Behring Diagnostics (Somerville, N. J.). Specific rabbit antisera to human trypsin and chymotrypsin were prepared as previously described (22, 24).

ENZYMATIC ACTIVITIES

The hydrolysis of BAEE (0.05 M in 0.15 M NaCl) at 37° was followed by an autotitrator (Radiometer Co., model SBR C2) with a 0.25 ml autoburette (ABU 12) and combined electrode (GK 2302C) using 0.05 M NaOH. The specific activity is expressed as micromoles per hr per mg protein and is referred to as arginine esterase (AE) activity.

The enzymatic activity towards BAPNA was determined spectrophotometrically (Beckman DB with 1 cm light path) according to Erlanger *et al.* (6).

The residual enzymatic activity of the immunoprecipitin line towards BAEE was performed as previously described for human trypsin immunoprecipitates (22). The activity of these precipitin lines towards BANA was determined as follows. Four milligrams of BANA and 40 mg *o*-dianisidine were dissolved in 0.5 ml dimethyl-formamide and 9.5 ml warmed (45°) phosphate buffer 0.2 M, pH 7.6, were added. This substrate solution was applied on the washed plate containing the immunoprecipitate at 38° for 1–3

hr in a humid chamber. Activity was observed when dark brown discoloration occurred.

POLYACRYLAMIDE SLAB GEL ELECTROFOCUSING

Polyacrylamide gel electrofocusing was performed at 4° with dual vertical slab gel, 3 mm thickness (Bio-Rad model 220) in 7.5% acrylamide (C = 2.5%) (Eastman-Kodak) containing 1% ampholine (LKB, pH range 4–8), with constant voltage (150 V) for 16 hr. A marginal strip was cut from both sides of the slab and the pH gradient was measured in 1-cm intervals. When the slabs were stained for protein, they were first immersed overnight in 10% trichloroacetic acid and stained with 1% amido black in 10% acetic acid.

When this procedure was used for the preparation of protein fractions, the slab was scanned at 280 nm with a scanning densitometer (Transidyne General, Ann Arbor, Mich.) and the protein bands removed. The acryl band with the protein fraction was homogenized with a glass hand homogenizer, dispersed in 3 ml saline, and dialyzed overnight against saline. The acrylamide was removed by centrifugation and the protein content determined according to the method of Lowry *et al.* (14).

IMMUNOLOGIC PROCEDURES

Immunization. Two rabbits were each immunized with 2 mg protein antigen in complete Freund's adjuvant (Difco) at multiple intradermal sites. Starting 10 days after immunization, the rabbits were bled weekly from the marginal ear vein. Antibody content of some of the individual bleedings was determined by a quantitative precipitin test (10) and was found to be 2.0–2.5 mg/ml. Antibody titer remained at this level for several months without the need for a booster injection. The antisera were pooled and the IgG fraction was prepared according to the method of Levy and Sober (13).

Double diffusion in agar gel, 1% in phosphate-buffered saline (PBS), was carried out according to the procedure of Ouchterlony (17). The plates were washed exhaustively with PBS, pH 7.4, the precipitin lines were photographed, and the residual enzymatic activity was demonstrated towards either BAEE or BANA. Immunoelectrophoresis was carried out in 0.05 M barbital buffer, pH 8.6, using agarose-coated sheets (Bioware, Inc., Wichita, Kan.) with a constant voltage of 8 V/cm for 2.5 hr. The plates were washed exhaustively in PBS and the precipitin arc stained for either protein by amido black or for residual enzymatic activity towards BANA.

Cross immunoelectrophoresis was performed according to the method of Laurell (12) as follows. The IgG fraction of the antiserum was added to 40 ml 0.75% agarose in 0.05 M barbital buffer, pH 8.6, cooled to 48° to a final concentration of 0.2 mg/ml (or in some experiments, 0.15 mg/ml). The solution was gently mixed and poured on glass plates (10 by 20 cm) and stored at 4° overnight. Wells (3.2 mm diameter) 1 cm apart were punched out and the samples (7 μl) applied. Electrophoresis with a constant voltage of 6 V/cm was performed using the same buffer for 12 hr. The plates were washed for 24 hr against PBS and the residual enzymatic activity towards BANA was determined.

α_2-MACROGLOBULIN DETERMINATION

The immunologic determination was carried out by single radial immunodiffusion according to Mancini *et al.* (15) in a fashion similar to that previously described for trypsin quantitation (22). The anti-α_2-macroglobulin antiserum final concentration in the agarose was 2%. The plasma samples applied for determination were diluted v/v with PBS and 6 μl were applied in duplicate. Four serial dilutions of pooled serum with a known α_2-macroglobulin concentration were used for the standard curve. The diffusion was completed at 38°. The washed plates were stained for residual BAEE activity. The concentration as reflected by bovine trypsin binding was carried out according to the method of Gannot (7) and expressed as micromoles of BAPNA hydrolyzed per hr by trypsin bound to 1 mg sample protein.

SOYBEAN TRYPSIN INHIBITOR-AFFINITY CHROMATOGRAPHY

Ten grams of STI (crude) were covalently bound to 400 g CNBr-activated Sepharose 4B according to the method of Axen *et al.* (1). The binding yields in different preparations were found to be 65–75%. The affinity adsorbent was washed exhaustively on a Buchner funnel with PBS until no protein could be detected when following absorbance of the eluates at 280 nm. The protein fraction obtained after fractionation at 50% $(NH_4)_2SO_4$ was dialyzed against PBS. Then 1,200 ml (protein, 60 mg/ml) were added to the semidry cake and stirred overnight at 4°. The nonadsorbed fraction was separated on a Buchner funnel and the cake washed first with 4 liters PBS and 6 liters 0.1 M phosphate buffer, pH 7.0, containing 0.4 M NaCl. The absorbance of the effluent at 280 nm after the washing was less than 0.02. The adsorbed fraction was eluted by stirring the adsorbent at 38° with 500 ml of the last buffer solution containing 0.5 M benzamidine. The eluate was dialyzed for 24 hr against saline and concentrated by negative pressure ultrafiltration.

ACTIVATED PLASMA FRACTIONS

Blood samples obtained from 15 healthy individuals and 15 cystic fibrosis patients were activated by chloroform-ellagic acid and handled as previously described (19). An equal volume of saturated $(NH_4)_2SO_4$ solution, pH 7.0, was added and the samples stirred and kept at 4° overnight. The precipitate separated by centrifugation was washed three times with 50% saturation $(NH_4)_2SO_4$ solution, suspended in a minimal volume of distilled water, and dialyzed at 4° for 48 hr against PBS. The samples were centrifuged at 22,000 × *g* for 30 min. After determination of the enzymatic activity towards BAEE, the samples were stored at −20°. Pooled normal plasma from healthy blood donors was obtained from blood banks and activated in a similar fashion.

Table 1. *Hydrolysis of α-N-benzoyl-L-arginine ethyl ester-HCl (BAEE) by various fractions of pooled normal plasma*

Plasma fractions	Total protein, g	BAEE, μmol hydrolyzed/hr/mg protein		
		pH 5.5	pH 6.5	pH 7.4
Activated plasma	134.68	0.20	0.35	0.41
50% saturation $(NH_4)_2SO_4$ precipitate (AE I)	73.75	0.08	0.41	0.51
Eluted from STI[1] affinity chromatography (AE II)	0.27	9.83	22.15	33.10

[1] STI: soybean trypsin inhibitor; AE: arginine esterase.

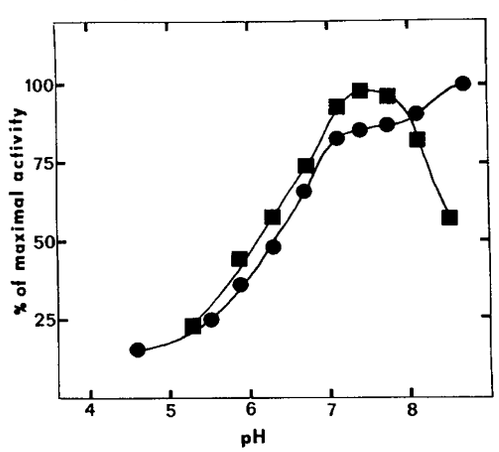

Fig. 1. ●——● represents the pH profile of partially purified arginine esterase (AE II fraction). ■——■ represents the preparation after incubation for 30 min at 55°.

RESULTS

PREPARATION AND CHARACTERIZATION OF ARGININE ESTERASES FROM POOLED NORMAL PLASMA

Normal plasma from nine healthy blood donors (2,600 ml) was activated by chloroform-ellagic acid treatment. An aliquot (10 ml) was dialyzed against saline for further studies and the remainder precipitated by $(NH_4)_2SO_4$ at 50% saturation, pH 7.0. The precipitate was washed with 50% $(NH_4)_2SO_4$ solution and dialyzed exhaustively against PBS. This fraction of the activated plasma was designated as AE I. The fraction was applied for affinity chromatography on 7 g STI insolubilized on Sepharose 4B and eluted with 0.5 M benzamidine. The fraction obtained by STI affinity chromatography was designated as AE II. The protein content and the enzymatic activity towards BAEE of the three fractions is summarized in Table 1. The heterogeneity of the enzymatic activity in all fractions is evident from the different

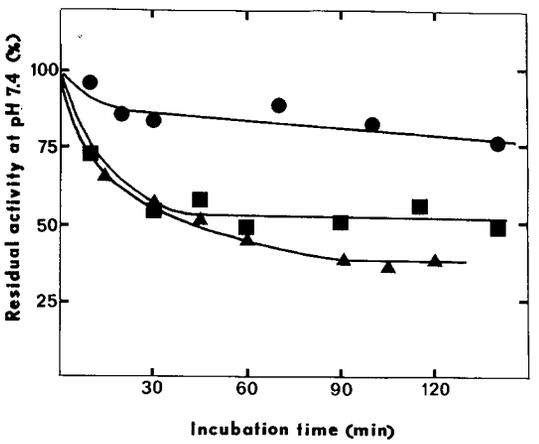

Fig. 2. Residual activity of partially purified arginine esterase (AE II fraction) after incubation at 42° (●——●); 48° (■——■); and 55° (▲——▲).

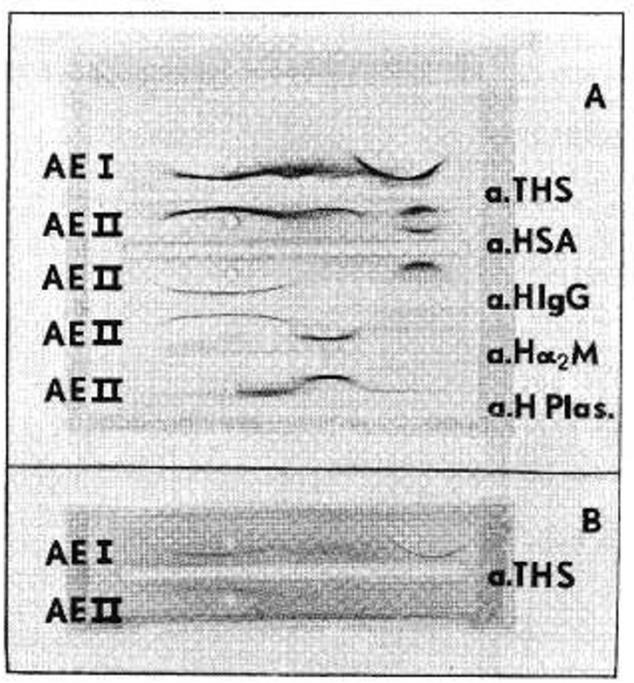

Fig. 3. *A:* immunoelectrophoresis of $(NH_4)_2SO_4$ precipitate of activated plasma AE I and of partially purified arginine esterase AE II with the following antisera: anti-human serum proteins (*a.THS*); anti-albumin (*a.HSA*); anti-IgG (*a.HIgG*); anti-α_2-macroglobulin (*a.H*α_2*M*); and anti-plasminogen (*a.HPlas.*). *B:* residual α-*N*-benzoyl-DL-arginine-β-naphthylamide-HCl activity in some of the immunoprecipitates.

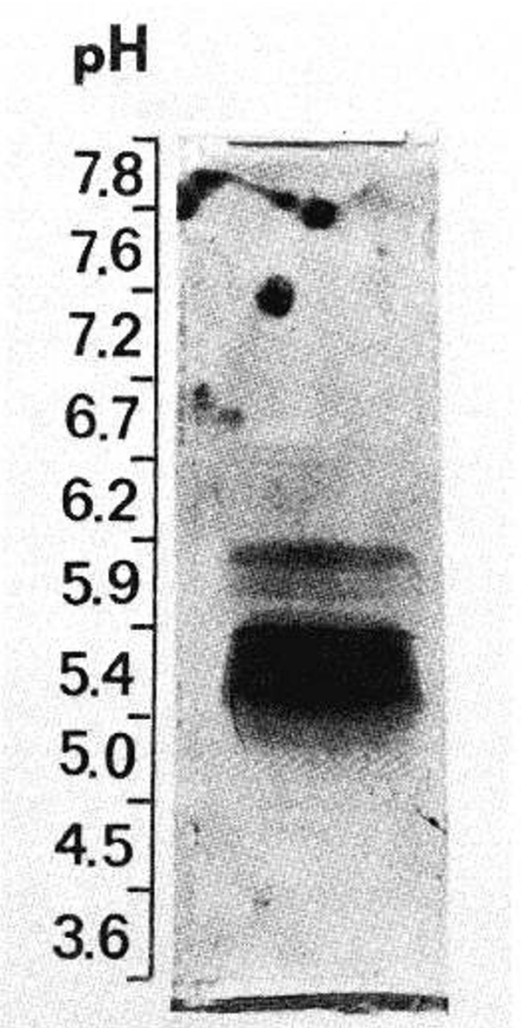

Fig. 4. Protein bands demonstrated after polyacrylamide slab gel electrofocusing of partially purified arginine esterase (AE II fraction). The pH of the various fractions is designated on the left side of the figure.

increases of the specific activity when determined at various pH values.

The pH profile of the partially purified AE preparation (AE II) is shown in Figure 1. A bimodal profile was obtained with one pH optimum at pH 7.4 and the second at pH above 8.5. In an attempt to separate the various enzyme components of AE II, this protein fraction was subjected to heat inactivation. The results are shown in Figures 1 and 2. It is shown that the enzyme component with the more basic pH optimum was more heat labile whereas the component with a pH optimum of pH 7.4 was relatively heat stable. No apparent inhibition of AE II activity towards BAEE was observed by *p*-hydroxymercuribenzoate at concentrations of 10^{-4}–10^{-3} M whereas STI (100 μg/ml) inhibited 87% of the activity.

AE II was examined by double gel diffusion against monospecific antisera to human trypsin, chymotrypsin, plasminogen, prothrombin, and C1 esterases, all proteases with arginine esterase activity. An antigenically cross-reacting material was shown only with the antisera to plasminogen. To rule out the possibility that enzymes might be present in the AE II fraction as complexes with various serum protease inhibitors, it was examined by double gel diffusion against antisera to α_1-trypsin inhibitor, inter-α-trypsin inhibitor, and α_2-macroglobulin. Only the anti-α_2-macroglobulin antiserum gave an immunoprecipitate with residual enzymatic activity towards both BAEE as well as towards BANA.

Further characterization of the protein components of AE II was obtained by immunoelectrophoresis with different antisera. As seen in Figure 3*A*, precipitin arcs were observed with anti-human

serum albumin, anti-human IgG, anti-human α_2-macroglobulin, and anti-human plasminogen. In Figure 3*B*, the residual enzymatic activity of some of these arcs is shown.

PREPARATION OF ANTIARGININE ESTERASE ANTISERUM

When the AE II protein fraction was subjected to isoelectrofocusing on polyacrylamide slab gel, numerous protein components were observed (Fig. 4). Twenty milligrams of AE II were applied for preparative polyacrylamide slab isoelectrofocusing. When the slab was scanned at 280 nm, four very close protein bands were shown in the area of pH 5.5. The acrylamide band containing this fraction was removed and used for immunization. The antiserum thus obtained was designated as anti-AE. The IgG fraction of this antiserum was prepared. The antiserum (anti-AE) was examined against AE II and compared with antisera against human IgG and human α_2-macroglobulin by double gel diffusion (Fig. 5). A precipitin line forming a spur formation with the other antisera and revealing enzymatic activity was observed.

When activated plasma samples of normal individuals and cystic fibrosis patients were examined by immunoelectrophoresis with this antiserum, four precipitin arcs with enzymatic activity were shown in the healthy donor samples whereas only three were shown in the cystic fibrosis samples (Fig. 6). As seen in this figure, the IgG arc with residual enzymatic activity was faint for both the cystic fibrosis and normal plasma samples. The arc missing in the cystic fibrosis samples is the one corresponding to the α_2-macroglobulin of Figure 3.

COMPARISON OF NORMAL AND CYSTIC FIBROSIS-ACTIVATED PLASMA SAMPLES

Fifteen plasma samples from healthy donors and 15 from cystic fibrosis patients were activated and the 50% saturation $(NH_4)_2SO_4$ fraction prepared. The enzymatic activity towards BAEE is summarized in Table 2. As observed before, this activity had components with different heat stability. Therefore, the fractions were compared with their residual activity after heat inactivation. As shown in the table, the samples from patients with cystic fibrosis revealed a lower enzymatic activity that was relatively heat stable as compared with normal but marked overlaps were found.

Since the activity missing in cystic fibrosis was associated with the enzyme-α_2-macroglobulin complex, we investigated the possibility that "enzyme-free" α_2-macroglobulin might be present in higher concentration in the activated plasma fractions from cystic fibrosis samples. In order to measure this component, the binding of bovine trypsin to free α_2-macroglobulin was determined by measuring its residual activity towards BAPNA in the presence of STI (trypsin protein esterase concentration). As shown in Table 2, the mean value of both α_2-macroglobulin and trypsin protein esterase concentration in the cystic fibrosis group was relatively higher. When the ratio of α_2-macroglobulin determinations by both procedures was calculated (Table 2), a lower ratio was shown in the cystic fibrosis group. Moreover, when these plates were washed exhaustively and the residual enzymatic activity towards BAEE of the precipitin rings was examined, marked qualitative differences were observed. Although both the normal and cystic fibrosis precipitin rings had some residual activity, the activity

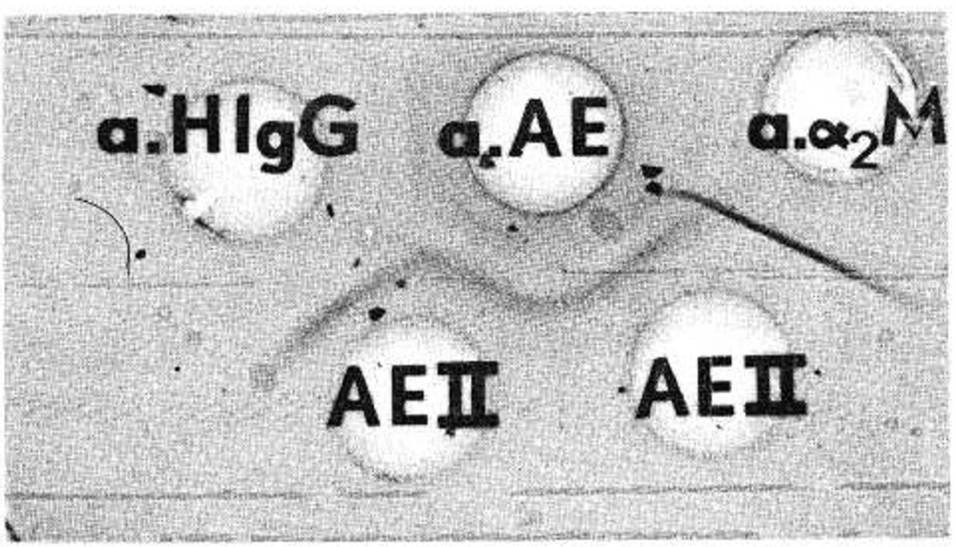

Fig. 5. Residual enzymatic activity (α-*N*-benzoyl-DL-arginine-β-napthylamide-HCl) of the immunoprecipitates obtained by double gel diffusion of partially purified arginine esterase. The antisera are designated as in Figure 3.

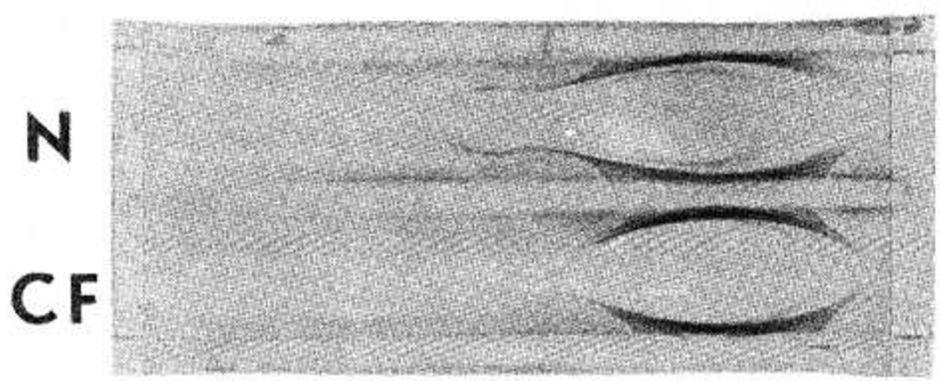

Fig. 6. Residual enzymatic activity (α-*N*-benzoyl-DL-arginine-β-napthylamide-HCl) of the immunoprecipitates obtained by immunoelectrophoresis of normal (*N*) and cystic fibrosis (*CF*) $(NH_4)_2SO_4$ precipitates of activated plasma. Anti-AE II has been applied to the slots.

Table 2. *Comparison of normal and cystic fibrosis-activated plasma fractions*[1]

	Hydrolysis of BAEE at pH 7.4 (specific activity)	Heat inactivation (%) after 30 min at 55°	Trypsin protein esterase concentration[2] (A)	α_2-Macroglobulin, mg/ml[3] (B)	A/B ratio
Normal	1.43 (1.1–1.7)	66 (48–76)	98.7 (61–144)	1.1 (0.88–1.52)	90
Cystic fibrosis	0.88 (0.6–1.3)	41 (34–54)	129.6 (78–171)	2.0 (1.44–2.44)	65

[1] BAEE: α-*N*-benzoyl-L-arginine ethyl ester-HCl. Values are expressed as means and ranges (parentheses).
[2] Micromoles of α-*N*-benzoyl-DL-arginine-*p*-nitroanilide-HCl hydrolyzed per hr by trypsin bound to 1 mg sample protein.
[3] Immunologic determination.

staining was fainter and appeared later in the rings of cystic fibrosis samples.

As shown previously, an immunoprecipitin arc with enzymatic activity was missing on immunoelectrophoresis in the cystic fibrosis samples (Fig. 6). The reproducibility of this observation in a double blind study revealed subjective problems in the definite documentation and made it impractical for use in individual clinical samples. Distinction between cystic fibrosis and normal became obvious by using immunoelectrophoresis in agarose containing antibodies (cross immunoelectrophoresis). Using this technique with agarose containing 0.2 mg/ml anti-AE IgG fraction, three coinciding "rockets" were observed when the washed plates were stained for BANA enzymatic activity. The usual symmetry observed in such "rockets" as well as the correlation between concentration and rocket length was not obtained with our enzymatic activity staining. One of these "rockets" was missing in the cystic fibrosis samples (Fig. 7*A*). In lower antibody concentrations (0.15 mg IgG/ml) only that one "rocket" missing in cystic fibrosis could be shown (Fig. 7*B*). A faint "rocket" of immunoprecipitate with no residual enzymatic activity was seen in the cystic fibrosis samples. When 15 normal samples and 15 cystic fibrosis samples were examined in a double blind fashion, 13 out of 15 healthy individual samples demonstrated this "rocket," whereas in 14 out of 15 cystic fibrosis samples, it was absent.

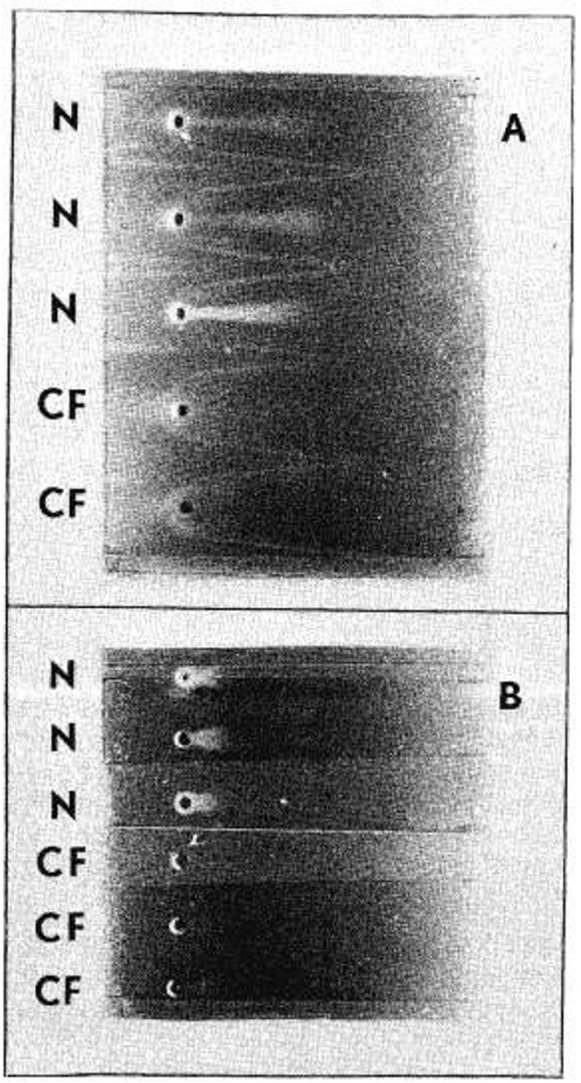

Fig. 7. Residual enzymatic activity (α-*N*-benzoyl-DL-arginine-β-naphthylamide-HCl) of immunoprecipitates obtained after electrophoresis of normal (*N*) and cystic fibrosis (*CF*)-activated plasma in agarose containing anti-AE II antibodies. In the upper plate (*A*), the concentration of the IgG fraction of anti-AE II was 0.2 mg/ml agarose. The concentration in plate *B* was 0.15 mg/ml agarose.

DISCUSSION

The present study using an immunologic methodology confirms previous observations from our laboratory of an absence of a protease component with arginine esterase activity in plasma of patients with cystic fibrosis (19, 20). Since previous studies had shown that this enzyme fraction is STI inhibited (19–21), we utilized affinity chromatography on STI as the approach for partial purification. The protein fraction obtained from activated plasma contained only one recognizable proteolytic enzyme, plasmin, in detectable concentrations (Fig. 3). The three other protein components with arginine esterase activity cross-reacted antigenically with commercially available antisera to human serum albumin, IgG, and α_2-macroglobulin. This partially purified arginine esterase preparation (AE II) was subjected to isoelectrofocusing (Fig. 4) and the protein fraction, pI 5.5, was used for immunization. The antiserum thus obtained revealed three distinct immunoprecipitates with residual enzymatic activity corresponding to those previously mentioned. No antibodies directed against plasmin could be demonstrated in this antiserum (anti-AE). The precipitin arc with residual enzymatic activity identified as serum albumin can be explained by the diversity of its nonspecific ligand binding affinities (11). Thus, such complexes could be adsorbed to the STI chromatography by the proteolytic enzyme specificity. We presume that the second precipitin arc with arginine esterase activity that is antigenically cross-reacting with IgG might represent kallikrein. Since the two proteins, IgG and kallikrein, are so closely associated, antiserum prepared against the purified enzyme had to be adsorbed with Fletcher factor-deficient plasma or with purified IgG (2). The third antigenic component in AE II with an isoelectric point of 5.5 was found to be antigenically an α_2-macroglobulin (Fig. 3*A*) that exhibits arginine esterase activity (Figs. 3*B* and 5). This enzymatic activity associated with an α_2-macroglobulin complex was shown to be deficient by both immunoelectrophoresis (Fig. 6) and immunoelectrophoresis in agarose containing antibodies (Fig. 7) in 14 out of 15 cystic fibrosis sera.

Recently, Wilson and Fudenberg (26), using multiple chromatographies, gel filtration, and starch block electrophoresis, documented that a protein fraction with a pI of 5.5, α_2-macroglobulin, was absent in plasma from cystic fibrosis patients. As in our observation, they also found a normal concentration of antigenically cross-reacting α_2-macroglobulin in cystic fibrosis and slightly reduced binding capacity for trypsin (Table 2). They suggested that this protein is an α_2-macroglobulin "proteolytic subunit derivative" and not a plasma protease as "plasma proteases do not have pI values near 5.5." However, in our study, the plasma fraction with a pI of 5.5 was obtained by its protease specificity toward the inhibitor STI. The residual enzymatic activity of this protein component in an immune complex with its specific antibodies could be demonstrated (Figs. 3, 4, and 7). Based on this observation, we suggest that the observed phenomenon of a missing protein with pI of 5.5 is secondary to an absence of an α_2-macroglobulin-protease complex or an α_2-macroglobulin subunit-protease complex. The spur formation observed with AE II antigens in double gel diffusion against the anti-AE antiserum and the commercial anti-α_2-macroglobulin (Fig. 5) indicates that only some of the antigenic determinants of α_2-macroglobulin were immunogenically expressed when this fraction was used for immunization. This observation suggests that in the α_2-macroglobulin-protease complex a number of antigenic determinants were altered and, therefore, the possibility of α_2-macroglobulin subunits is likely.

It was shown that α_2-macroglobulin (3, 8, 9) binds and inhibits essentially all endopeptidases in approximately equimolar ratio and does not react with exopeptidases and nonproteolytic hydrolases. The proteolytic enzyme cleaves a peptide bond in the α_2-macroglobulin molecule irreversibly. An entrapped α_2-macroglobulin presumably binds only proteases in their active enzyme form (3). The present study and that of Wilson and Fudenberg (26) demonstrate no difference in α_2-macroglobulin concentration in plasma of control subjects and patients with cystic fibrosis. Therefore, the study is consistent with the hypothesis that the absence of the α_2-macroglobulin-protease complex reflects a molecular defect in either of the two components as originally suggested by Wilson and Fudenberg (26). More recent studies in our laboratory have shown that the α_2-macroglobulin molecule is altered in patients with cystic fibrosis and obligate heterozygotes (23).

SUMMARY

The present study using immunologic methodology confirms previous observations from our laboratory of an absence of a protease component with arginine esterase activity in plasma of patients with cystic fibrosis. Arginine esterase in plasma from normal individuals was partially characterized, subjected to isoelectrofocusing, and the plasma fraction with a pI of 5.5 used for the preparation of antisera. The antisera obtained revealed four distinct immunoprecipitates with residual enzymatic activity in normal subjects, whereas only three immunoprecipitates were seen in plasma of cystic fibrosis patients. The precipitin arcs with the residual enzymatic activity were identified. One arc with enzymatic activity was found to be antigenically an α_2-macroglobulin. The enzymatic activity associated with the α_2-macroglobulin complex was shown to be deficient by both immunoelectrophoresis and immunoelectrophoresis with agarose containing antibodies in 14 out of 15 patients with cystic fibrosis. In contrast, in 13 of 15 normal individuals, this α_2-macroglobulin-protease complex was readily demonstrated. These results are consistent with our earlier demonstration of a deficiency of proteolytic activity in cystic fibrosis and suggest that this activity is apparently complexed with α_2-macroglobulin.

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