

Cat allergen 1: Biochemical, antigenic, and allergenic properties

Kathleen Leitermann, S.B., and John L. Ohman, Jr., M.D. Boston, Mass.

Cat allergen 1, an important agent in human allergic reactions, has been partially purified by affinity chromatography. Heating the purified allergen at 100° C for 30 min resulted in a 28% loss in the antigenicity of the allergen molecule (determined by Laurell rocket assay), although lower temperatures had little effect. Its allergenicity (determined by passive transfer skin test) was diminished slightly after heating to 56° or 100° C. Reduction with dithiothreitol or 2-mercaptoethanol resulted in greater losses of antigenicity and allergenicity but did not obliterate these properties. Three forms of the affinity-purified allergen (isoallergens) differing slightly in isoelectric point were demonstrated by isoelectric focusing followed by crossed immunoelectrophoresis. The molecular weight of cat allergen 1 under physiologic conditions was $35,000 \pm 2000$ as determined by gel filtration in Sephadex G-75. Under the dissociative conditions of sodium dodecyl sulfate-polyacrylamide gel electrophoresis, with or without prior reduction by dithiothreitol, cat allergen 1 appeared to exist as an antigenically active subunit with a molecular weight of $18,000 \pm 2000$. This subunit molecular weight estimate was confirmed by gel filtration in 6M guanidine hydrochloride. The stability of the allergenic and antigenic activity of cat allergen 1 suggests that this activity may be determined partially by the primary sequence of allergenic sites on the molecule. The separation and purification of molecular subunits may allow sequence analysis of these sites. (J ALLERGY CLIN IMMUNOL 74:147-53, 1984.)

Allergens are a class of antigen that give rise to allergic disease in humans. They are known to have extremely high biologic potency; as little as 10^{-13} gram can induce a positive wheal-and-flare response in skin.¹ Studies of the general properties of allergens, reviewed elsewhere,^{1, 2} have demonstrated that most major allergens are proteins with apparent MWs between 20,000 and 40,000 and that most allergens lose their antigenic activity upon denaturation.

Allergens derived from the domestic cat frequently cause severe allergic reactions including bronchial asthma.³ Studies from different parts of the world have documented sensitization to cat allergens in 20% to 30% of patients with asthma.⁴⁻⁷ A single allergen, found on cat pelts and designated cat allergen 1, has

Abbreviations used

MW:	Molecular weight
CPE:	Cat-pelt extract
pI:	Isoelectric point
DEAE:	Diethylaminoethyl
DTT:	Dithiothreitol
PAGE:	Polyacrylamide gel electrophoresis
BSA:	Bovine serum albumin
OVA:	Ovalbumin
CT:	Chymotrypsinogen
Cyto c:	Cytochrome c
PBS:	Phosphate-buffered saline (0.01M, pH 7.5; 0.15M NaCl)
SDS:	Sodium dodecyl sulfate
PBS-Tween:	PBS containing 0.1% Tween 20
Tris:	Tris(hydroxymethyl)aminomethane

From the Allergy Research Laboratory, Veterans Administration Outpatient Clinic, and Tufts University School of Medicine, Boston, Mass.

Supported in part by the Veterans Administration Medical Research Service.

Received for publication Sept. 16, 1983.

Accepted for publication Feb. 5, 1984.

Reprint requests: John L. Ohman, Jr., M.D., VA Outpatient Clinic, 17 Court St., Boston, MA 02108.

been implicated as a major cause of these reactions.^{8, 9} This article describes the properties of cat allergen 1 obtained from CPE by affinity chromatography. Under physiologic conditions cat allergen 1 has a MW of approximately 35,000 and exists in three forms that differ slightly in pI. Under dissociative conditions the allergen has an apparent MW of 18,000 and retains

its ability to precipitate with monospecific antiserum to cat allergen 1. Reduction of disulfide residues was not required for dissociation of this molecular subunit, indicating that it is not covalently linked.

MATERIAL AND METHODS

Material

DEAE-cellulose (DE52) was obtained from Whatman Chemical Separation Inc. (Clifton, N.J.). Sephadex G-75 and G-100, cyanogen bromide-activated Sepharose 4B, and blue dextran were obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Biogel P-100 was obtained from Bio-Rad (Rockville Centre, N. Y.). Acrylamide, agarose, Coomassie blue stains (G-250 and R-250), and DTT were obtained from either Bio-Rad or LKB Productor (Bromma, Sweden). An LKB Multiphor was used for Laurell rocket immunoelectrophoresis, analytical isoelectric focusing, and crossed immunoelectrophoresis; a Bio-Rad System 220 was used for PAGE. Power for all electrophoresis and focusing was supplied by an LKB 2300 power supply. Complete Freund's adjuvant was obtained from Difco Laboratories (Detroit, Mich.). BSA, bovine gamma globulin, OVA, CT, and Cyto c were from Sigma Chemical Co. (St. Louis, Mo.). For the RAST inhibition assay, Immulon-coated Remov-a-well microtiter plates from Dynatech (Alexandria, Va.) and a Beckman Instruments, Inc. (Somerset, N. J.) gamma counter were used. All other chemicals were of reagent grade.

Preparation of acetone precipitated CPE

Unshaven cat pelts were rinsed with water, lyophilized, and slurried with water (1:10, w/v) with periodic adjustment to pH 7.5 by use of 0.1M NaOH, for 6 hr at room temperature and then allowed to stand overnight at 4° C. The supernatant was filtered through dacron mesh, clarified by centrifugation (2000 × g, 15 min), and lyophilized. This CPE was dissolved in 80% acetone (aqueous, v/v), stirred for 1 hr, and centrifuged. The precipitate was washed twice in 80% acetone, resuspended in water, dialyzed against a 0.01M phosphate buffer, pH 7.0 (100 vol), and clarified by centrifugation.

Production of anti-cat allergen 1 and anti-cat albumin antisera

Rabbit antiserum to cat allergen 1 and to cat albumin were obtained as previously described.⁹ Sheep antiserum to cat allergen 1 was obtained from Dr. Harold Baer.¹⁰ The antigen was purified from acetone-precipitated CPE by use of DEAE-cellulose ion-exchange chromatography, Sephadex G-100 gel filtration (twice), and preparative PAGE. Protein eluted from a single band in the final electrophoresis step was emulsified with complete Freund's adjuvant,¹¹ for initial immunizations, and with incomplete Freund's adjuvant for boost immunizations approximately 1 mo later. Each serum produced a single precipitin line against CPE in Ouchterlony double-diffusion experiments. The precipitin lines produced by rabbit anti-cat allergen 1 and sheep anti-cat allergen 1 also fused in a pattern of complete identity.

Laurell rocket assay of cat allergen 1 and cat albumin

Cat allergen 1 and cat albumin were assayed by quantitative immunoelectrophoresis according to the method of Laurell.¹² Monospecific antiserum was mixed with liquid 1% agarose at 56° C and poured to form a 2-millimeter thick gel layer (183 by 102 mm). Plates were placed on a cooling platform and connected by paper wicks to electrophoresis buffer reservoirs. Serial dilutions of a standard CPE (149) and assay samples were applied to wells cut into the gel on each plate. Approximately 10 V/cm was applied across the gel for 3 to 4 hr. Gel plates were dried and stained with Coomassie blue G-250.¹³ The log heights of the resulting precipitin arcs were directly proportional to the log concentration of antigen in the samples, and a standard curve, constructed by use of this relationship, was used to estimate antigen concentrations in unknown samples. The concentration of cat albumin was expressed in micrograms per milliliter and that of cat allergen 1 was expressed in units per milliliter (U/ml) in accordance with the standards for cat allergen 1 from Dr. Harold Baer of the Bureau of Biologics, Food and Drug Administration. The correspondence of units to micrograms of protein is not known.

Total protein determination

Total protein concentrations were determined by comparison of optical densities at 280 nm in 0.1M NaOH of test solutions with those of standard solutions of BSA; standards were serial dilutions of a 1.0 mg/ml of stock prepared with 25 mg desiccated BSA powder.

Affinity purification of cat allergen 1

An affinity column for the preparation of purified anti-cat allergen 1 antibody was prepared by (1) coupling cat allergen 1 (obtained by DEAE-cellulose and Sephadex chromatography as previously described⁹) to cyanogen bromide-activated Sepharose 4B (8 ml),¹⁴ (2) stirring the washed slurry overnight with an excess of human serum albumin at 4° C and (3) equilibrating with PBS in a 2.2 by 3.0 cm column. Affinity-purified antibody was obtained by applying sheep anti-cat allergen 1 antiserum (6 to 8 ml) to the column, washing with PBS to remove unadsorbed proteins (100 ml, which proved to reduce the optical density at 280 nm of eluate to 0), and eluting with glycine HCl-buffered saline (0.065M, pH 2.4; 0.15M NaCl). The antibody-containing eluate was immediately neutralized with 0.2M of Tris, and the column was washed with 25% acetic acid (100 ml) that was followed by PBS (200 ml). Thirteen separations (i.e., 90 ml sheep antiserum) yielded 13.3 mg purified anti-cat allergen 1 as determined by absorbance at 280 nm in 0.1M NaOH.¹⁵

The purified anti-cat allergen 1 antibody was used to prepare the immunosorbent for allergen purification by coupling it to cyanogen bromide-activated Sepharose 4B (10 ml).¹⁴ Affinity-purified cat allergen 1 was obtained from the immunosorbent column after applying acetone-precipitated CPE (6 to 8 ml, 180 to 240 U), washing with PBS, and eluting as described above.

Estimation of cat allergen 1 MW by gel filtration

A Sephadex G-75 column (1.6 by 90 cm) was equilibrated at room temperature with PBS. Affinity-purified cat allergen 1 (20 U) and MW standards (blue dextran, BSA, OVA, CT, and Cyto c, 1 to 5 mg each) were mixed in 0.5 ml of PBS. The sample was centrifuged to remove undissolved protein and applied to the column. Standard proteins were detected by absorbance at 280 nm in a flow cell, and allergen was detected by Laurell rocket assay. Allergen applied to this column in amounts less than 100 U did not produce detectable absorbance at 280 nm. The MW of the allergen was estimated from a plot of the log of MW versus (elution volume)/(void volume) (i.e., V/V_0) for the protein standards.¹⁶

Estimation of allergen subunit MW by SDS-PAGE and gel filtration

SDS-PAGE was performed according to the method of Laemmli,¹⁷ but without a stacking gel, in 17.5% acrylamide (2.6% bis-acrylamide) slab gels. Affinity-purified cat allergen 1 and standard proteins (OVA, CT, and Cyto c) were reduced in 0.125M Tris, pH 6.8, and 2.5% SDS with 0.125M DTT at room temperature for 30 min under N_2 . Reduced standards and cat allergen 1 as well as nonreduced samples of allergen in 0.125M of Tris, were applied to separate sample wells in the gel. After electrophoresis at 60 mA for 2 hr, gel tracts containing reduced and nonreduced allergen were excised for crossed immunoelectrophoresis.¹⁸ Acrylamide strips were embedded along the edge of an agarose gel containing anti-cat allergen 1 antiserum and subjected to gel electrophoresis at 10 V/cm for 3 hr. Agarose gels were dried and stained as described above. Duplicate allergen samples and standard proteins in the slab gel were fixed with 10% acetic acid and 45% methanol (v/v, aqueous), stained with 0.25% Coomassie blue R-250 in 10% acetic acid and 45% methanol (v/v) for 2 hr, and destained in 7% acetic acid and 5% methanol (v/v).

Dissociative gel filtration was performed with a Biogel P-100 column (1.5 by 55 cm) equilibrated with 6M guanidine-HCl. A sample containing affinity-purified cat allergen 1 (11 U) and MW standards of bovine gamma globulin, CT, sperm whale myoglobin, Cyto c, insulin, and tyrosine (0.5 to 1 mg each) dissolved in 6M guanidine-HCl was applied to the column. Standard proteins were detected by absorbance at 280 nm, and allergen was detected by Laurell rocket assay on fractions that had been dialyzed against 250 vol of distilled water. The MW of the dissociated allergen was estimated from the V/V_0 plot as above.

Determination of allergenic activity by the passive transfer test

One tenth ml of serum from a cat-sensitive patient was injected intradermally into multiple sites on the upper arm of one of the investigators who was previously demonstrated to have negative skin tests to cat antigens. The serum had been previously demonstrated to sensitize nonatopic human skin to cat allergen 1 but not to cat albumin. After a

latent period of 48 hr, the sensitized sites were challenged with cat-allergen samples by prick test.⁹ The average diameter of the resulting wheal was measured after 15 min.

Thermal modification or reduction and alkylation of cat allergen 1

Solutions of allergen (12.4 U) in 0.75 ml of 0.55M Tris, pH 8.1, were heated to 37°, 56°, or 100° C for 30 min and cooled to 0° C for 1 hr. A control solution was kept at room temperature before cooling. Identical solutions were reduced with DTT (0.125M) or 2-mercaptoethanol (0.75M) for 30 min, sparged intermittently with N_2 , and alkylated with iodoacetic acid (1.0M) with adjustment to pH 8.5 with 0.2M NaOH for 1 hr at 0° C. All samples were dialyzed against 0.05M NH_4HCO_3 and adjusted to a volume of 2.0 ml. Serial 1/4 dilutions of each solution were prepared in PBS for passive transfer testing.

RAST inhibition

A RAST-inhibition assay was performed by use of a modification of the microtiter solid phase radioimmunoassay.¹⁹ Affinity-purified cat allergen 1 was placed in microtiter wells (0.1 ml/well, 0.37 U/ml in PBS) and incubated 4 hr at 4° C. The wells were emptied, refilled with 0.1% (w/v) human serum albumin in PBS (0.2 ml/well), and incubated for 1 hr at 4° C. The wells were emptied and washed three times with cold PBS-Tween.

A pool of defibrinated plasma from two individuals who were clinically sensitive to cats was mixed 1:1 with serial 1/2 dilutions of the hyperimmune animal antiserum to be tested. Aliquots (0.1 ml) of these mixtures were added to the coated microtiter wells (in duplicate) and allowed to equilibrate at 4° C overnight. After washing three times with cold PBS-Tween, ¹²⁵I-labeled anti-human IgE (4 ng in 0.1 ml PBS-Tween) was added to each well and allowed to equilibrate for 22 hr at room temperature. Wells were again washed three times with PBS-Tween and dried. Radioactive iodine bound to individual wells was detected (at 30 to 160 KeV) in a gamma spectrometer. Percent inhibition was calculated with the formula:

$$\% \text{ Inhibition} = 100 - 100 \frac{X - B}{S - B}$$

X = (average cpm of duplicate inhibited-serum wells) - (environmental background (EB))

B = (average cpm of duplicate wells with non-specifically bound ¹²⁵I-labeled anti-IgE) - (EB) (In these experiments B = O)

S = (average cpm of duplicate uninhibited-serum wells) - (EB)

Analytical isoelectric focusing

Analytical isoelectric focusing was performed in 7% polyacrylamide (2.6% bis-acrylamide) gels containing 2% ampholytes (pI 2.5 to 9.5). Samples containing 1.3 to 13 U of cat allergen 1 (in 0.01 to 0.07 ml) were applied to pieces of filter paper (5 by 10 mm) laid onto the gel surface and focused at 25 W for 150 to 165 min. Gel tracts containing focused proteins were treated in one of the following ways:

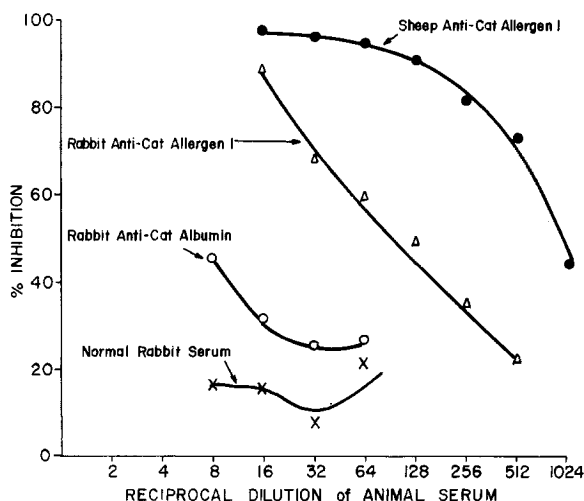


FIG. 1. Inhibition of binding of human IgE to cat allergen by hyperimmune animal antisera (RAST inhibition). Dilutions of animal antisera in PBS were mixed with human cat-allergic defibrinated plasma and incubated in microtiter wells coated with cat allergen 1. Wells were washed and bound human IgE detected by incubating with ^{125} I-labeled anti-IgE, washing, and determining cpm bound to the wells. Inhibition was calculated as the percent reduction in cpm bound over background as compared to the uninhibited human plasma cpm bound.

1. Focused proteins in a gel tract containing 13 U of cat allergen 1 were fixed in 45% methanol (v/v) containing 11.4% trichloroacetic acid and 3.4% sulphosalicylic acid (w/v). The gels were stained and destained as described above.

2. Gel tracts (5 mm wide) were divided into 19 sections (5 by 5 mm). Each section was eluted overnight in 1.0 ml deionized water, and the pH of each eluate was determined. These solutions were also tested for allergenicity by the passive transfer skin test.

3. Crossed immunoelectrophoresis was performed as described for SDS-PAGE.

RESULTS

Affinity purification of cat allergen 1

Cat allergen 1 was partially purified from crude CPE by use of the specific cat 1 immunoabsorbent. An average yield of 37% of the cat allergen 1 applied to the column was obtained, and the ratio of cat allergen 1 to total protein in the eluate was sevenfold to tenfold higher in the eluate than in the CPE applied. In each of three experiments, the pelt extract applied to the immunoabsorbent contained >10 mg of cat albumin, whereas <1% of this albumin contaminated the purified eluate.

Stability of the allergenic and antigenic properties of the allergen

Heated or reduced and alkylated samples of affinity-purified cat allergen 1 were assayed for antigenicity by Laurell rocket immunoelectrophoresis

TABLE I. Stability of the allergenic and antigenic activity of cat allergen 1

Procedure	Allergenic activity* dilution producing a wheal of ≥ 3 mm	Antigenic activity U/ml Cat Allergen 1 (Laurell rocket assay)
20° C, 30 min	1/64	6.1
37° C, 30 min	1/64	6.0
56° C, 30 min	1/16	5.7
100° C, 30 min	1/16	4.4
Reduction in 0.125M DDT and alkylation†	1	1.1
Reduction in 0.75M 2 ME‡ and alkylation	1/4	1.5

*Passive transfer technique: 0.1 ml of serum from a cat-allergen subject was injected into marked skin sites of a normal subject. After 48 hr the sites were challenged with dilutions (1, 1/4, 1/16, 1/64) of affinity-purified cat allergen 1 by prick test.

†Samples were alkylated with 1.0M iodoacetic acid, pH 8.5, 0° C, for 1 hr.

‡2 ME = 2-mercaptoethanol.

and for allergenicity by passive transfer prick test. As summarized in Table I, heating at 37° or 56° C had little effect on the antigenicity, but at 56° C, the allergenicity of cat allergen 1 was diminished. Heating to 100° C for 30 min resulted in a 28% loss of antigenicity with a concomitant diminution in allergenicity of cat allergen 1. A much higher loss of antigenicity (75%) and allergenicity resulted from reduction with 2-mercaptoethanol, whereas the highest loss of both antigenicity (82%) and allergenicity resulted from reduction with DTT.

The measurement of allergenic activity by the passive transfer test depends on the presence of human IgE antibody, whereas the measurement of antigenic activity depends on the presence of precipitating antibody in rabbit or sheep antiserum. The binding affinities of human, rabbit, and sheep antibody were compared by the RAST-inhibition technique. As illustrated in Fig. 1, the rabbit anti-cat allergen 1 and sheep anti-cat allergen 1 both competed effectively with human IgE for binding sites on the allergen. In comparison, normal rabbit serum and rabbit anti-cat albumin inhibited binding to a small degree.

Demonstration of isoallergens

Affinity-purified cat allergen 1 was subjected to analytical isoelectric focusing (Fig. 2). The pH gradient formed in the gel extended from 2.47 to 8.47. Crossed electrophoresis revealed three well-defined arcs joined with a precipitin line of complete identity in the pH range of 3.0 to 4.2 with the major precipitin

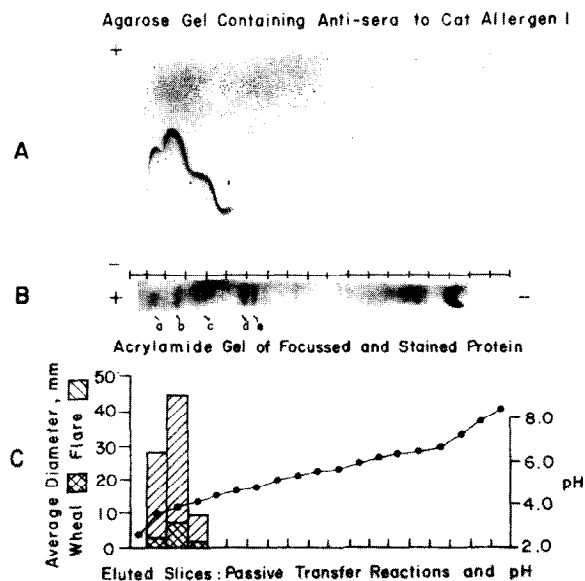


FIG. 2. Demonstration of isoallergens. Affinity-purified cat allergen 1 was subjected to isoelectric focusing in acrylamide followed by crossed immunoelectrophoresis. Illustrated are a stained acrylamide gel tract containing 13 U of cat allergen 1 and the precipitin arcs formed in the agarose crossed immunoelectrophoresis gel of 1.3 U of allergen focused in the same acrylamide slab. Prick test reactions to gel slice eluates in passively sensitized skin are recorded as mean diameters of the wheal (induration)/flare (erythema) appearing at the test site after 15 min. The pH gradient in the gel was determined from gel slices eluted in 1.0 ml distilled water overnight.

arc at a pI of 3.85. The stained gel tract revealed two faintly stained protein bands denoted *a* and *b*, and a less well-defined area, *c*, that corresponded to these three precipitin arcs. In addition, (albumin band *d*) at pI 4.5 (identified by a crossed electrophoresis that is not illustrated), another protein (band *e*) at pI 4.6, and several protein bands in the pI range of 5.8 to 8.5 are also observed. Allergenic activity was detected by the passive transfer test in eluate from slices that contained proteins denoted *a*, *b*, and *c*. Band *b*, which corresponded to the major precipitin arc, was also associated with the highest allergenic activity as determined by the passive transfer test. No antigenic or allergenic activity was detected in any other section of the gel. Isoelectric focusing of affinity-purified cat allergen 1 followed by crossed electrophoresis was repeated six times, and nearly identical patterns of fused precipitin arcs and stained protein bands were observed.

MW of the allergen under physiologic conditions

Affinity-purified cat allergen 1 was subjected to column chromatography in PBS on a Sephadex G-75 column and quantified by Laurell rocket assay of

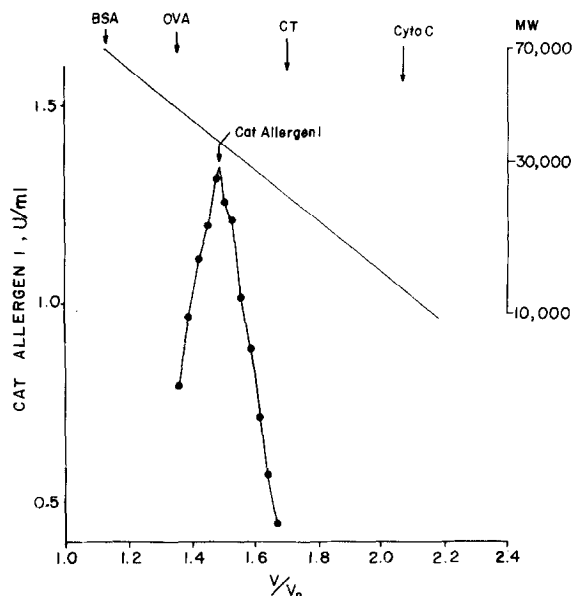


FIG. 3. Estimation of the MW of cat allergen 1 under associative conditions. Affinity-purified cat allergen 1 (20 U) was mixed with blue dextran, BSA, OVA, CT, and Cyto c and chromatographed in PBS. The elution volumes of blue dextran (V_o) and protein standards (V) were determined by detection of optical density at 280 nm. Cat allergen 1 was measured in eluate fractions by the Laurell rocket assay (dl = lower detection limit of assay).

eluted fractions. Fig. 3 demonstrates the elution profiles of the allergen with the V/V_o values for standard proteins (BSA, OVA, CT and Cyto c) run simultaneously. From the V/V_o versus log molecular weight standard curve generated by the standard proteins and the V/V_o of the peak of eluted allergen, the apparent molecular weight of cat allergen 1 is estimated at $35,000 \pm 2000$ daltons under physiologic conditions.

MW of the allergen under dissociative conditions

An estimation of the MW of affinity-purified cat allergen 1 under dissociative conditions was made by SDS-PAGE. Both reduced and nonreduced samples of the allergen, as well as reduced standard proteins, were subjected to electrophoresis. The allergen was identified by crossed immunoelectrophoresis into an agarose gel containing monospecific antiserum to cat allergen 1. Fig. 4 demonstrates the precipitin arcs formed in the agarose gel, aligned above their corresponding Coomassie blue-stained SDS-polyacrylamide gels. The nonreduced allergen (Fig. 2, B and C) and reduced allergen (Fig. 2, D and E) migrated to approximately the same position in the gel ($\pm 10\%$). R_f values for the standard proteins were plotted against the log of their MWs to produce the standard curve demonstrated (Fig. 2, A). Cat allergen 1 has an

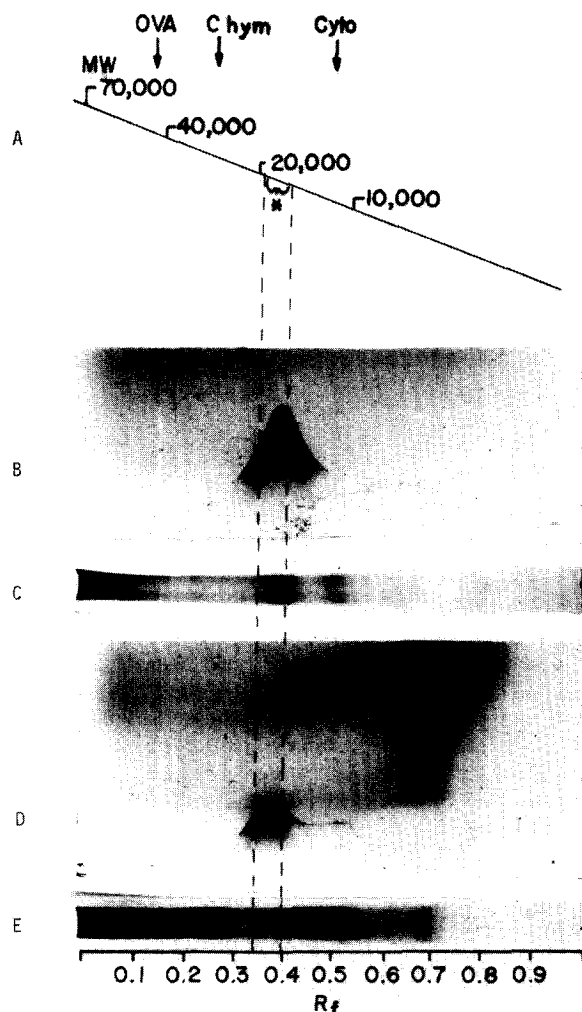


Fig. 4. Estimation of the MW of cat allergen 1 by SDS-PAGE. Affinity-purified cat allergen 1 was subjected to SDS-PAGE (17.5% acrylamide gel) and crossed immunoelectrophoresis after incubation with SDS alone (non-reduced, **B** and **C**) or with SDS and DTT (reduced, **D** and **E**). Protein standards OVA, CT, and Cyto c were run separately in the same gel slab to produce the standard curve (**A**).

apparent MW of $18,000 \pm 2000$, estimated from the average R_f values of those points on the acrylamide gels corresponding to the peaks of the crossed immunoelectrophoretic precipitin arcs. The Coomassie blue-stained SDS-PAGE gels reveal several contaminating protein bands associated with nonreduced and reduced allergen (Fig. 2, **C** and **E**, respectively). Although most of these are clearly separated from detectable allergen, one protein comigrates with allergen in the nonreduced state, although not in the reduced state.

To confirm the findings of SDS-PAGE, estimation of the MW of the allergen under the dissociative influence of 6M guanidine-HCl was made. Affinity-

purified cat allergen 1 was subjected to chromatography on a Biogel p-100 column equilibrated with 6M guanidine-HCl and quantified by Laurell rocket assay of eluted fractions. From the V/V_0 versus log MW standard curve generated by the standard proteins run in the same sample, the apparent MW of cat allergen 1 is $17,500 \pm 2000$ (data not demonstrated).

DISCUSSION

This study used affinity chromatography to partially purify cat allergen 1 from crude CPE. The probable reason this method has not been used in the past for allergen purification is that, for most allergenic substances studied, the major allergen is one of the more abundant proteins in an aqueous extract.¹ Most extracts of cat pelt, however, contain a very small amount of cat allergen 1⁸ and large amounts of albumin and other proteins. The high degree of specificity of the cat allergen 1 immunoabsorbent allows rapid, single step, partial purification of cat allergen 1, which we have found to be of a purity comparable to that obtained by sequential ion exchange and Sephadex chromatography.⁹

Heating or reduction and alkylation diminished the antigenicity of cat allergen 1 as measured by Laurell rocket immunoelectrophoresis, as well as its allergenicity as measured by the passive transfer test (Table I), although these properties were not obliterated. Irreversible conformational or chemical changes induced in the allergen protein by heat denaturation may alter or destroy some of its antigenic or allergenic sites. Similarly, the effect of reduction and alkylation may be to disrupt intrachain disulfide bonds or to induce an undefined chemical modification unrelated to disruption of disulfide linkages. Despite these changes, however, some allergenic sites remain intact, suggesting that they may be determined by the primary sequence of the molecules.

Isoelectric focusing of affinity-purified allergen reveals the presence of three forms of the allergen (i.e., isoallergens) differing in pI yet recognized as the same antigen by anti-cat allergen 1 antisera. These allergenically active forms occurred in the pH range 3.5 to 4.1. The same pI range of allergenicity was observed by Varga and Ceska²⁰ for crude cat extracts. The isoallergens of cat allergen 1 are distinct from cat albumin, which was reported by Stokes and Turner²¹ to have minor allergenicity. The observed pI differences for the cat isoallergens may be attributable to protein microheterogeneity or to attachment of carbohydrate.

Cat allergen 1 has an apparent MW of approximately 35,000 when fractionated on Sephadex G-75 under physiologic conditions. Its apparent MW under

the dissociative conditions of SDS-PAGE is $18,000 \pm 2000$, but reduction with 0.125M DTT does not affect this estimate. These data suggest that the native allergen comprises two subunits that are not covalently linked because reduction does not affect the apparent MW in SDS-PAGE. The allergen subunit retained its ability to react with anti-cat allergen 1 antisera after dissociation, although it is not known whether it reassociated during the crossed immunoelectrophoresis procedures to which it was subjected before meeting the antibody. Whether or not the subunits reassociate, the relative stability of the antigenic sites to dissociative treatments again suggest that they may be determined partially by the primary sequence of the molecule.

In summary, we have purified cat allergen 1 by affinity chromatography. Three slightly different isoallergens appear to exist with pIs that differ approximately 0.2 to 0.3 pH units. The apparent MW of cat allergen 1 under the dissociative influence of 6M guanidine-HCl or SDS is approximately half of its apparent MW under physiologic conditions, suggesting that the allergen consists of two polypeptide chains and, since reduction with DTT does not affect the SDS-PAGE MW estimate, that these chains are not disulfide linked. In order to produce allergic disease, an allergen must penetrate the mucosal barrier of the respiratory tract. Cat allergen 1 might be particularly prone to mucosal penetration because of its ability to dissociate into low MW fragments that remain antigenically active. The stability of allergenic sites on the molecule is indicated by their ability to cause passive transfer reactions after thermal denaturation or reduction and alkylation. These properties bode well for the localization and identification of allergenic sequences of the molecule that may be applicable to immunotherapy.

REFERENCES

- King TP: Chemical and biological properties of some atopic allergens. *Adv Immunol* 23:77, 1976
- King TP: Immunochemical properties of some atopic allergens. *J ALLERGY CLIN IMMUNOL* 64:159, 1979
- Skin tests and clinical features of asthma. A report to the Research Committee of the British Thoracic and Tuberculosis Society. *Br J Dis Chest* 69:125, 1974
- Bryant DH, Burns MW: Skin test reactions to inhalant allergens in asthmatic patients. *Med J Aust* 1:918, 1976
- Sarsfield JK, Boyle AG, Rowell EM, Moriarty SC: Pet sensitivities in asthmatic children. *Arch Dis Child* 51:186, 1976
- Kurata JH, Glovsky MM, Newcomb RL, Easton JG: A multifactorial study of patients with asthma. Part 2: Air pollution, animal dander and asthma symptoms. *Ann Allergy* 37:398, 1976
- Taylor WW, Ohman JL Jr, Lowell FC: Double-blind trial of immunotherapy in cat-induced asthma. *J ALLERGY CLIN IMMUNOL* 61:283, 1978
- Ohman JL Jr, Lowell FC, Bloch KJ, Kendall S: Allergens of mammalian origin. V. Properties of extracts derived from the domestic cat. *Clin Allergy* 6:419, 1976
- Ohman JL Jr, Lowell FC, Bloch KJ: Allergens of mammalian origin. III. Properties of a major feline allergen. *J Immunol* 113:1668, 1974
- Anderson MC, Baer H: Allergically active components of cat allergen extracts. *J Immunol* 127:972, 1981
- Freund J, McDermott K: Sensitization to horse serum by means of adjuvants. *Proc Soc Exp Biol Med* 49:548, 1942
- Laurell CB: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal Biochem* 15:45, 1966
- Fazekas De St. Groth S, Webster RG, Datyner A: Two new staining procedures for quantitative estimation of proteins on electrophoretic strips. *Biochim Biophys Acta* 71:377, 1963
- Axen R, Porath J, Ernback S: Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. *Nature* 214:1302, 1967
- Little JR, Donahue H: Appendix I. Spectral properties of proteins and small molecules of immunological interest. In Williams CA, Chase MW, editors: *Methods in immunology and immunochemistry*. New York, 1968, Academic Press, p 346
- Whitaker JR: Determination of molecular weights of proteins by gel filtration. *Anal Chem* 35:1950, 1963
- Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680, 1970
- Laurell CB: Antigen-antibody crossed electrophoresis. *Anal Biochem* 10:358, 1965
- Reid MJ, Cheung NV, Lewiston NJ: Microtiter solid-phase radioimmunoassay for specific immunoglobulin E. *J ALLERGY CLIN IMMUNOL* 67:263, 1981
- Varga JM, Ceska M: Characterization of allergen extracts by polyacrylamide gel isoelectrofocusing and radioimmunosorbent allergen assay. II. Dog and cat allergens. *Int Arch Allergy Apply Immunol* 42:438, 1972
- Stokes CR, Turner MW: Isolation and characterization of cat allergens. *Clin Allergy* 5:241, 1975