

Identification, Cloning, and Recombinant Expression of Procalin, a Major Triatomine Allergen¹

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Among the most frequent anaphylactic reactions to insects are those attributed to reduviid bugs. We report the purification and identification of the major salivary allergen of these insects. This 20-kDa protein (procalin) is a member of the lipocalin family, which includes salivary allergens from other invertebrates and mammals. An expression system capable of producing reagent quantities of recombinant allergen was developed in *Saccharomyces cerevisiae*. Antisera produced against recombinant protein cross-reacts with ELISA with salivary allergen. Recombinant Ag is also shown to react with sera from an allergic patient but not with control sera. By immunolocalization, the source of the salivary Ag is the salivary gland epithelium and its secretions. *The Journal of Immunology*, 2001, 167: 2694–2699.

Among the most frequently reported anaphylactic reactions to biting insects are those attributed to a small but medically important group of hematophagous bugs that comprise the subfamily Triatominae (Heteroptera:Reduviidae) (1). Although best known as arthropod vectors of Chagas disease, these insects also inject salivary proteins during the acquisition of a blood meal that may initiate a variety of severe and occasionally fatal allergic responses in sensitized individuals. Systemic reactions include generalized pruritis, gastrointestinal disturbances, fever, dyspnea, syncope, hypotension, laryngeal and glossal edema, and convulsions (2–6). Extreme hypersensitivity resulting in death has been attributed to the bite of *Triatoma protracta* (6).

In the U.S., allergic reactions have been associated with *T. protracta*, *T. rubida*, *T. recurva*, *T. sanguisuga*, *T. gerstaeckeri*, and *Paratriatoma hirsuta* (6, 7). Allergic sensitization, demonstrated by anti-*Triatoma* IgE Ab, may develop in as many as 7% of individuals residing within the range of these insects (7). The expansion in both seasonal and perennial human incursions into chaparral or woodland habitats of *T. protracta* in the western U.S. has increased the number of persons at risk for *Triatoma* hypersensitivity; it is estimated that as many as 30,000 persons in California are at risk for anaphylaxis from this insect (7).

Isolation of proteins from the saliva of several species of Triatominae, and characterization of the antihemostatic properties of these proteins, has been the subject of many recent investigations (8–13). However, little is known about the molecular identity of salivary allergens of these insects. Initial studies with *T. protracta*

indicate that ~89% of the allergenic activity in the saliva of this bug represents reaction to a 18- to 20-kDa protein (14). We now report the purification of this major allergenic protein and isolation of a cDNA clone. Successful expression of recombinant Ag in yeast provides reagent quantities of Ag for subsequent investigations of the serologic diagnosis, epidemiology, and desensitization therapy of individuals at risk of severe allergic reaction to the bite of *T. protracta*. We also predict the functional and primary allergenic residues in procalin through sequence analysis and structural models.

Materials and Methods

Ag purification and amino-terminal sequence analysis

Paired salivary glands were dissected from 50 fourth-instar *T. protracta* nymphs and suspended in PBS (pH 7.4). Extracts were filtered through a 0.2- μ m cellulose acetate membrane, dialyzed with a 6-kDa cutoff, and initially fractionated by fast protein liquid chromatography on a MonoQ HR5/5 (Pharmacia, Uppsala, Sweden) cationic exchange column. The sample was loaded in 20 mM Tris-HCl (pH 7.4) and eluted with a NaCl gradient (0–1 M). One-milliliter fractions were collected from a flow rate of 1 ml/min. Three immunologically active fractions were identified by ELISA, using banked serum samples from patients with confirmed allergy to *T. protracta* (7), and a goat anti-human IgG-HRP conjugate (Boehringer Mannheim, Mannheim, Germany). Sera were obtained from four patients all of whom had at least three life-threatening episodes of anaphylaxis. Ag-containing fractions were purified by using an HPLC system (Rainin Instruments, Emeryville, CA) with a variable wavelength monitor (Knauer, Berlin, Germany). Ag-containing fractions were applied to a C8 peptide reverse-phase column (4.6 $\times$ 250 mm; Vydac, Hesperia, CA) in 0.05% trifluoroacetic acid (TFA³; Sigma, St. Louis, MO) and eluted with a 50% methanol/50% water/0.5% TFA gradient (0–100% in 30 min). One-milliliter fractions were collected and analyzed by ELISA and SDS-PAGE. The purified 20-kDa Ag was stored at 4°C for 24 h to allow the TFA to evaporate before Edman degradation for N-terminal sequence analysis by using a 470A protein sequencer (Applied Biosystems, Foster City, CA) with an on-line 120A phenylthiohydantoin analyzer (Applied Biosystems).

Isolation and cloning of *Triatoma* allergen cDNA

A 32-fold degenerate oligonucleotide primer (5'-ACAGAATTCCA(A/G)AA(A/G)CC(T/G)AA(A/G)CC(T/G)ATGGA-3') was deduced from amino acid residues 4–10 of the amino-terminal sequence of the purified Ag. RNA from two pairs of nymphal *T. protracta* salivary glands was extracted in RNazol B (Biotecx Laboratories, Houston, TX) and reverse

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³ Abbreviations used in this paper: TFA, trifluoroacetic acid; BLAST, basic local alignment search tool.

transcribed by using 400 U Moloney murine leukemia virus reverse transcriptase (Life Technologies, Gaithersburg, MD) and the primer 5'-ACAATCGATAAGCTTTTTTTTTTTTTTTT-3'. First-strand cDNA was amplified by using PCR. One micromolar of each of the above primers were used in a 50- μ l reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each dNTP, and 1.25 U *Taq* DNA polymerase (Boehringer Mannheim). A total of 45 cycles were performed by using a DNA thermal cycler (PerkinElmer Cetus, Norwalk, CT). The first two cycles were performed with annealing at 35°C for 2 min, denaturing at 94°C for 1 min, and extension at 72°C for 2 min. For the subsequent 43 cycles, annealing occurred at 50°C for 2 min, denaturing at 94°C for 1 min, and extension at 72°C for 2 min. The amplified product was gel-purified, electro-eluted in a Spectra/Por molecular porous dialysis membrane (Spectrum Medical Industries, Houston, TX), and subcloned into the *Eco*RI and *Hind*III sites of pBluescript (Stratagene, La Jolla, CA). The forward and reverse strands of the recombinant DNA were sequenced by the dideoxy chain termination method using T7 DNA polymerase (United States Biochemical, Cleveland, OH).

A 5' RACE was used to identify the upstream region of the message. First-strand cDNA was again produced by reverse transcription of salivary gland mRNA from two nymphs using 45 pM of a primer designed to the reverse strand at positions 446–463 (5'-TTGAAAGAATATAATGCC-3'). The cDNA was treated with 1 U *Escherichia coli* RNase H (Boehringer Mannheim) and purified in a GlassMAX spin cartridge (Life Technologies). An oligo(dC) anchor sequence was added to the 3' end of the cDNA using 0.4 U TdT (Boehringer Mannheim). The tailed fragment was amplified by using PCR, with 0.4 μ M of an oligo(dG) anchor primer (5'-ATA GAATTCGGGGGGGGGGGG-3') and 0.4 μ M of a nested primer designed to reverse-strand positions 422–438 (5'-ACAAAGCTTCTTGCCA GCATTAGGAC-3'). A 50- μ l reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each dNTP, and 1.25 U *Taq* DNA polymerase was amplified for 35 cycles with annealing at 50°C for 2 min, denaturing at 94°C for 1 min, and extension at 72°C for 2 min. The amplified fragment was gel purified, subcloned into the pT7Blue vector (Novagen, Madison, WI), and fully sequenced in both directions.

Expression of procalin cDNA in yeast

A cDNA coding for amino acids 4–151 was amplified with primers to remove the 3' untranslated region and incorporate *Xba* and *Sal*I restriction sites at the 5' and 3' ends, respectively. The resultant fragment was digested with *Xba* and *Sal*I and subcloned into the shuttle plasmid pAB125, creating a fusion gene containing the glucose-repressible alcohol dehydrogenase 2/glyceraldehyde-3-phosphate dehydrogenase promoter at the α factor leader sequence of *Saccharomyces cerevisiae* (15). The expression cassette was digested with *Bam*HI and *Sal*I and ligated into the yeast expression plasmid pBS24.1. An overnight culture of the AB 122 strain of *S. cerevisiae* carrying the *ura3* and *leu2* mutations was grown to OD₆₁₀ = 1.0. A 45- μ l aliquot of yeast was mixed with 2 μ g of pBS 24.1 containing the expression cassette and transformed by using an Electro Cell Manipulator 600 (BTX, San Diego, CA) at mode 2.5 kV, resistance 129 Ω , charging voltage 1.0 kV, pulse length 5 ms, and chamber gap 2 mm. Electroporated yeast was plated onto uracil-deficient SD medium, and transformants were subsequently transferred to L-leucine-deficient medium for final selection. Secreted recombinant protein was collected from the supernatant of yeast cultures grown for 72 h at 30°C in yeast extract peptone dextrose medium with 1% glucose. Recombinant protein eluted from a PD10 column equilibrated in 20 mM Tris (pH 7.4), was loaded at 3 ml/min onto a Q Sepharose Fastflow column (Amersham Pharmacia Biotech, Piscataway, NJ) equilibrated in 20 mM Tris (pH 7.4). The recombinant protein eluted in the flow through.

Determination of recombinant Ag immunogenicity

Polyclonal antiserum to the recombinant protein was raised in a female New Zealand white rabbit. A primary immunization of 500 μ g of purified lyophilized protein in CFA was followed by an i.m. booster injection containing 250 μ g protein in IFA at 3 wk. Hyperimmune rabbit serum was harvested at 7 wk. Native salivary gland and recombinant protein samples were run on a 13% SDS-PAGE gel, transferred to a polyvinylidene difluoride membrane, probed with a 1/500 dilution of hyperimmune rabbit serum, washed, and incubated with alkaline phosphatase-linked goat anti-rabbit IgG (1/1000). Ab-labeled proteins were visualized with 5-bromo-4-chloro-3-indolyl-1-phosphate/nitroblue tetrazolium (Promega, Madison, WI).

ELISA with human sera

Recombinant procalin (Tpa-2) was coated onto 96-well polystyrene enzyme immunoassay plates (Costar, Cambridge, MA) at 1 or 10 ng/ μ l in 50 μ l of PBS (0.001 M KH₂PO₄, 0.01 M Na₂HPO₄, 0.137 M NaCl, 0.0027 M KCl (pH 7.4)) at 4°C overnight. The wells were rinsed, and then the remaining sites were blocked by incubation at room temperature for 2 h with 100 μ l 0.05% Tween 20, 1% BSA in PBS. Serial dilutions of human sera from a known allergic patient (positive toward *T. protracta* extract) and control human sera were made in blocking buffer (after clearing sera by centrifugation for 4 min at 16,000 $\times g$). Then 50 μ l of each dilution was incubated with the recombinant Tpa-2-coated wells for 1 h at room temperature. The rinsed wells were then incubated for 1 h at room temperature with 100 μ l of an alkaline phosphatase-labeled secondary Ab to human IgGs, IgM made in goat (Zymed, South San Francisco, CA) at either 1/500 or 1/2000 dilution (in blocking buffer). The rinsed wells were then incubated 30 min at room temperature with alkaline phosphatase substrate (*p*-nitrophenylphosphate; Zymed), and the absorbance was measured in a spectrophotometric plate reader at 405 nm (Vmax; Molecular Devices, Menlo Park, CA).

Immunolocalization of salivary Ag of *T. protracta*

Immunohistochemical staining was performed using both intact *T. protracta* and separately dissected salivary glands. Whole bugs or isolated salivary glands were fixed in 8% paraformaldehyde-0.1 M phosphate buffer (pH 7.4) for 24 h, dehydrated, and embedded in methacrylate plastic (16). The entire procedure was conducted at 4°C. Sections (2.5 μ m) were cut and incubated overnight at 4°C with a 1/1000 dilution of hyperimmune rabbit antiserum reactive with the recombinant protein, which were washed and incubated with a 1/100 dilution of alkaline phosphatase-labeled goat anti-rabbit Ab by using the Vectastain ABC (avidin-biotin complex)-alkaline phosphatase kit (Vector Laboratories, Burlingame CA) (17, 18).

Results

Purification of major *T. protracta* allergen and isolation of a cDNA

Fig. 1 shows fractionation of the salivary gland proteins by MonoQ ion-exchange and HPLC CC8 chromatography as monitored by ELISA using pooled serum samples from patients with allergy to *T. protracta*. A single 20-kDa protein band corresponded to the purified allergen as identified by ELISA. Amino-terminal sequencing of this 20-kDa salivary gland Ag tentatively determined amino acid residues 1–20 as DE(?) (Q/E) (N/K) P(E/K) PM(Q/E) GFSATQF (H/Y) (K/Q) G. A 32-fold degenerate oligonucleotide primer corresponding to positions 4–10 (KQPKPME) was used in conjunction with an oligo(dT) primer to amplify a single DNA fragment of 517 bp from reverse-transcribed, polyadenylated RNA isolated from the salivary glands of *T. protracta*. This fragment was subcloned and sequenced, revealing an open reading frame encoding 138 aa downstream from the 4–10 primer. Primers were deduced from the internal sequence of the cloned fragment and used in a 5' RACE reaction to obtain the upstream region of the gene encoding the signal peptide and the first 10 amino acids of the sequenced protein.

The complete 611-bp cDNA sequence (GenBank accession number AF179004) contains an open reading frame of 507 bp encoding a protein with 169 amino acids (Fig. 2), with a predicted molecular mass of ~19 kDa. The partial amino acid sequence of the amino terminus obtained from Edman analysis matches the sequence predicted by the cDNA clone, except for a blank cycle produced by a cysteine in position 3 and the misidentification of threonine for serine in position 15. A hydrophobic 18-residue signal peptide sequence precedes the mature protein, with a predicted cleavage site corresponding to the site determined by N-terminal sequencing (19). The nucleotides preceding the start codon match the translation initiation sequence (A/C)AA(A/C)ATG described for other orders of Insecta (20), including genes for salivary gland proteins of hematophagous Diptera (21, 22) and Hemiptera (10,

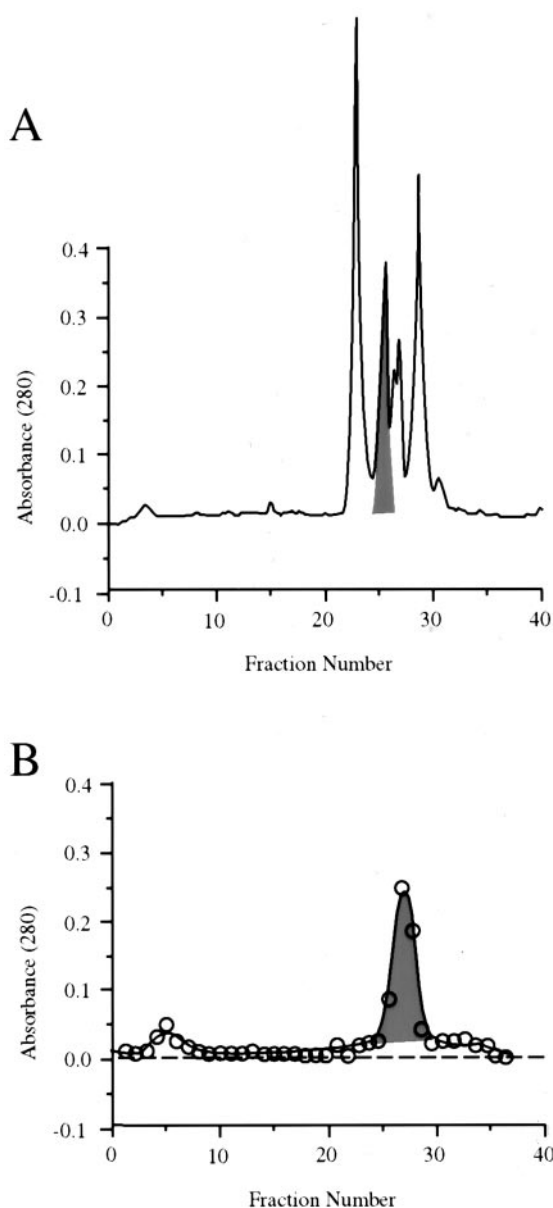


FIGURE 1. A, MonoQ purification of *T. protracta* allergen. Salivary gland extracts were prepared as described in *Materials and Methods*. One-milliliter fractions were analyzed by ELISA for reactivity with serum samples from allergic individuals (3). Positive ELISA is indicated by shading. Only fractions 25–27 (peak 2) had ELISA activity with known allergic patient sera and were pooled for reversed-phase HPLC purification. B, HPLC (C8 reversed phase) purification of ELISA-positive peak 2 from MonoQ fractionation. A single 20-kDa species was present in fractions 25–30, which contained all ELISA-positive protein.

12). No asparagine-linked glycosylation sites are identified. Comparison of the sequences produced by RT-PCR and by 5' RACE reveals nucleotide ambiguities at seven positions, resulting in six amino acid substitutions.

The deduced amino acid sequence reveals homology with several members of a family of extracellular transport proteins termed lipocalins (23, 24). Basic local alignment search tool (BLAST) searches using each of the possible procalin sequences identify homologies between procalin and the two sequences for the salivary platelet inhibitor from *Rhodnius prolixus* and the five sequences for triabin, the thrombin inhibitor from the saliva of *Tri-*

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1  TAGATCCAAAGTAAATTAACCTCGCCAACATGAAGACGTTTCATTGTAATC
-18                               MetLysThrPheIleValIle

52  ACATTATTTGGAATCCTGTCTACGCATATGCTGACGAATGTGAAAATCCA
-11  ThrPheIleGlyIleLeuSerTyrAlaTyrAlaAspGluCysGluAsnPro

103  GAACCTATGCAAGGTTTGTAGTGCCTCACAATTTTACCAAGGARAGTGGTAT
7    GluProMetGlnGlyPheSerAlaSerGlnPheTyrGlnGlyLysTrpTyr
    (Glu)

154  GTRACTCATGAACATCCGCAWCAACTCTATCCGAATGTAACATTCTTACT
24   ValThrHisGluThrSerAlaSerThrLeuSerGluCysAsnIleLeuThr
    (Thr)

205  ACTAGCAATGATAATGGCAAATTCAGTGTAAACATAAAATATACCAAAGAT
41   ThrSerAsnAspAsnGlyLysPheThrValLysHisLysTyrThrLysAsp

256  GGAAAWGTGGGTGAACTCATTTGTGAGGGTCAAGCAAGCGCAAATAATAAA
58   GlyLysValGlyGluLeuIleCysGluGlyGlnAlaSerAlaAsnAsnLys
    (Asn)

307  TTTACTTACGACTGCAAAATTTGRCGGASAGACTATGGAGCAAGTAACTCGA
75   PheThrTyrAspCysLysPheAspGlyGluThrMetGluGlnValThrArg
    (Gly) (Gln)

358  ACAGCAATGGATACAGACTATAATGACTACGCTCTGTACTATTATGTACA
92   ThrAlaMetAspThrAspTyrAsnAspTyrAlaLeuTyrTyrLeuCysThr

409  ACTTATAAAWATGGTCTTAATGCTGGCAAGAAAGAGGGCATTATATCTT
109  ThrTyrLysAsnGlyProAsnAlaGlyLysLysGluGlyHisTyrIleLeu
    (Tyr)

460  TCAAGAAGACAACCAAAATACAGAAATTCCTGATGCGCTGAAAATAAGACA
126  SerArgArgGlnProAsnThrGluIleProAspAlaLeuLysThrLysThr

511  AAGGATTTGAATTTGAAATTATGTGGTTAAATACAAAGACATTTTTTGT
143  LysAspLeuAsnLeuLysLeuCysGly

562  TGAAGTGATTATTTTAAATAAACCGACATTTAAAAA

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FIGURE 2. Sequence of the 19-kDa salivary allergen gene of *T. protracta*. Nucleotide ambiguities are noted at positions 145 (A/G), 156 (A/G), 175 (A/T), 261 (A/T), 329 (A/G), 334 (G/C), and 418 (A/T). Amino-terminal sequence of purified allergen is underlined. The pattern -G-X-W- shared by all members of the lipocalin protein family is bold.

atoma pallidipennis (12, 25), or its precursor at E-values of less than 1.e-5, the cutoff for significant homology in BLAST searches. Pallidipin precursor, a platelet inhibitor precursor found in the saliva from *T. pallidipennis* (10), and a human alipoprotein occasionally had BLAST scores close to but not above 1.e-5.

The combination of secondary structure predictions and threading results indicate that procalin is a lipocalin. PhD predicts that procalin is composed of eight extended β strands and a helix at the C terminus, a motif consistent with the lipocalin superfamily. Threading template structures that yield statistically significant matches to procalin are all lipocalins. From the diverse 123D database containing 1101 domains, only three structures (bilin binding protein (1bbpA), retinol binding protein (1aqb), and β -lactoglobulin (1bebA)) match with significant statistical weight. All three are lipocalins. Similar results are obtained from the hybrid threading algorithm of Fischer, with the lipocalin triabin as the highest scoring threaded structure by a large margin.

The best aligned sequence with procalin comes from an isoform of the salivary platelet aggregation inhibitor from *R. prolixus*. This sequence has no known structure from which a model can be constructed. The second highest match was to the triabin precursor sequence. Triabin, but not its precursor, has been solved crystallographically (25). The pro domain of triabin and procalin are nearly identical and hence the active solution form of procalin likely is homologous to triabin.

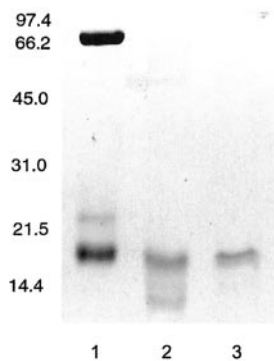


FIGURE 3. SDS-PAGE analysis of recombinant salivary Ag (procalin), visualized by Coomassie brilliant blue. *Lane 1*, Native *T. protracta* salivary gland extract (10 µg); *lane 2*, unpurified recombinant protein (70 µl concentrate); and *lane 3*, purified recombinant procalin. N-terminal sequencing (10 cycles) of the excised 17-kDa band (by gel migration) matched the corresponding residues predicted by the cDNA clone (19 kDa by amino acid molecular mass prediction).

Expression of recombinant protein in yeast

Secreted recombinant Ag (procalin) was collected for 72 h at 30°C in yeast extract peptone dextrose medium with 1% glucose, yielding 3.8 mg/100 ml culture medium after purification (Fig. 3). The

recombinant protein was transferred by immunoblotting to polyvinylidene difluoride membranes (Problott) from 16% SDS-PAGE (see above), and gas-phase sequencing revealed the amino-terminal sequence of the recombinant protein to be (?)PEPMQGF, consistent with the expected product. Immunoblot analysis using antisera raised against recombinant procalin confirmed a cross-reactivity with the native salivary gland allergen (Fig. 4A). Furthermore, serum from a highly allergic patient reacted with recombinant procalin on ELISA (Fig. 4B).

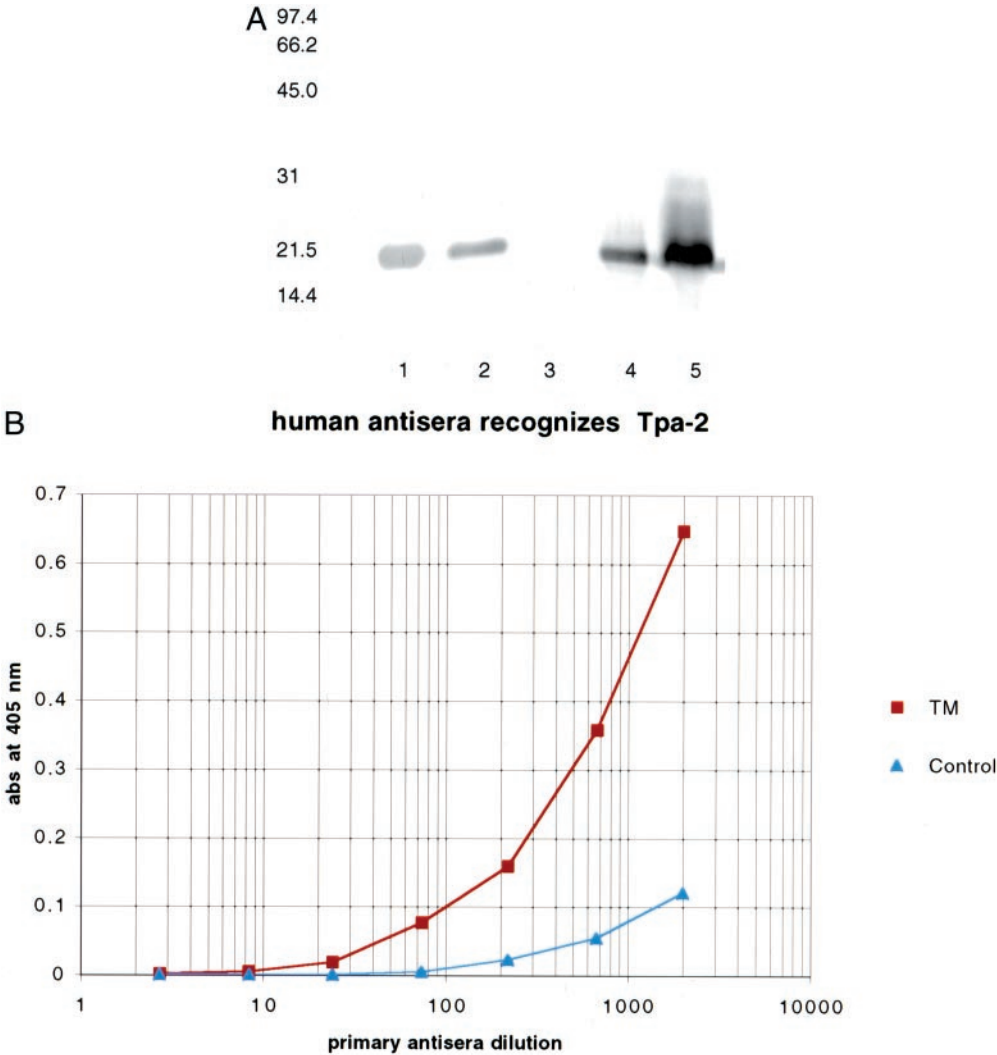
Immunolocalization of allergen in salivary gland cells and saliva

Fig. 5 shows immunohistochemical localization of procalin to the salivary glands of *T. protracta* using rabbit antiserum produced against purified recombinant procalin. Intense immunostaining was localized to the cytoplasm of simple cuboidal epithelium of the principle and accessory salivary glands (26) and to the luminal contents of the glands. Positive staining was confined to the salivary gland tissues and secretions of the insect and was not identified in any other tissue.

Discussion

Hematophagous bugs of the subfamily Triatominae may cause severe and occasionally fatal allergic reactions in sensitized persons. Anaphylaxis attributed to the bite of these insects has been reported from many regions of the Western Hemisphere, including

FIGURE 4. A, Immunoblotting of native and recombinant Ag with rabbit Ab raised to purified, recombinant procalin. *Lane 1*, Native *T. protracta* salivary gland extract; *lane 2*, TCA-precipitated *T. protracta* recombinant allergen (procalin); *lane 3*, blank; *lane 4*, purified recombinant procalin, TCA precipitated; and *lane 5*, lyophilized procalin. B, ELISA confirming reactivity of serum sample from highly allergic individual to recombinant procalin. TM (■), Allergic individual; control (▲), pooled sera from nonallergic individuals.



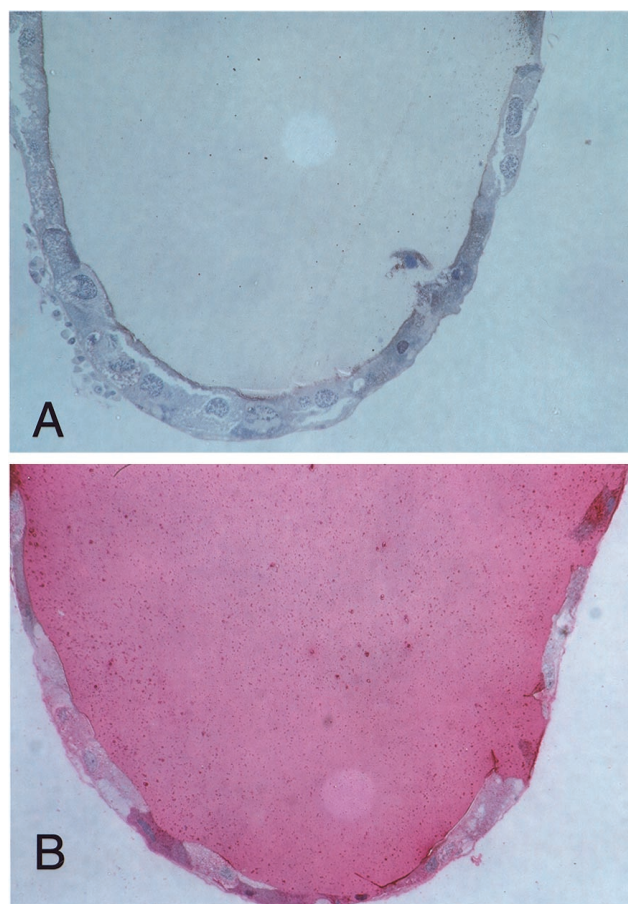


FIGURE 5. Immunolocalization of 19-kDa salivary allergen of *T. protracta*. Salivary glands or whole bugs were fixed, embedded, and sectioned as described in *Materials and Methods*. A, Control Ab; B, anti-procalin rabbit antisera (1:1000) as primary Ab. Alkaline phosphatase-linked secondary Ab (goat anti-rabbit) reaction localizes allergen to salivary gland epithelial cells and contents of salivary gland lumen (original magnification, $\times 200$).

the western and southwestern U.S., Hawaii, Argentina, Brazil, Chile, Bolivia, Peru, Ecuador, Uruguay, and Paraguay (6). Previous work identified salivary gland secretions of *T. protracta* as the source of the Ag(s), and an 18- to 20-kDa protein was identified as a major allergen (14). Using a serum bank of individuals with known allergy to this insect, an allergen was purified from salivary glands of *T. protracta* and a partial amino-terminal amino acid sequence obtained by Edman degradation. cDNA was synthesized from the mRNA of *T. protracta* salivary gland cells, and a PCR product was amplified by using an oligonucleotide based on the amino-terminal sequence of the purified allergen.

The protein allergen predicted from the cDNA clone shows homology to members the lipocalin family of proteins. Amino acid sequence identity among lipocalins is characteristically low, often as low as 10–20%. However, crystallographic analyses of several members of this protein family reveals a remarkably conserved three-dimensional structure: eight antiparallel β -strands that form a cup-shaped barrel around an internal ligand-binding site (23). This family is characterized by a heterogeneous group of small-secreted proteins with high affinity and selectivity for hydrophobic ligands. In this capacity, lipocalins may function in the extracellular transport of insoluble lipophilic molecules including retinoids, steroids, and small metabolites (27). Some lipocalins are reported to bind to specific cell surface receptors and may be

directly involved in cell regulation (23, 24) or synthetic pathways of PGs (28). Although the biologic functions of procalin, the 19-kDa salivary protein of *T. protracta*, are not specifically known, lipocalin-based proteins with distinct antihemostatic properties have been isolated from the salivary glands of related species of triatomine bugs, including *T. pallidipenis* and *R. prolixus* (25, 29). Both structural and sequential alignment methods predict that procalin is similar to triabin, an exosite thrombin inhibitor and procalin (12).

Several major allergens of animals and other insects have also recently been identified as lipocalins (30). These include urinary protein allergens of rodents, the canine allergens CANF1 and CANF2, the food allergen β -lactoglobulin, the bovine allergen BOSD5, and a cockroach allergen. As triggers of intermediate-type hypersensitivity reactions, lipocalins are known to bind IgE both in vitro and in vivo. T cell epitopes identified in the bovine lipocalin allergen have been shown to colocalize with conserved amino acid sequence motifs of the lipocalin family (30). The alignment of sequences at the C termini of Bos d 2 and procalin yielded a plausible prediction of the major epitope in procalin. The C-terminal region of both Bos d 2 and procalin have fairly high sequence homology, with residues at the very center of the putative allergenic site in procalin identical with the center of the known allergenic site in Bos d 2. In addition, the putative epitope in procalin shares a chemical signature with other allergens in the lipocalin family. In Bos d 2 and Bos d 5 (31), there appears to be a motif near the most allergenic regions that starts with a proline, followed by a few polar or positively charged amino acids, immediately before an isolated stretch of one to three hydrophobic residues, followed by another stretch of polar and charged residues ending with either a lysine or arginine. Every known allergen in the lipocalin family (32) has this motif within its sequence, although the motif's location relative to the C terminus varies considerably. Procalin's putative C terminus epitope also contains this motif (residues 130–139).

At least two different polymorphisms exist for the 19-kDa salivary Ag gene. Because mRNAs used to obtain these sequences were obtained from multiple bugs, it is not known whether these polymorphisms reflect multiple alleles within one insect or genetic heterogeneity among the insects from which the salivary glands were collected. Minor polymorphisms have also been observed among cDNA clones of other recently identified salivary proteins of triatomine bugs, including pallidipin (10) and triabin (12).

Isolation of a full-length cDNA coding for a 19-kDa salivary allergen of *T. protracta* allowed the subsequent heterologous expression of a recombinant protein (procalin) in *S. cerevisiae*. The vector chosen to express the allergen is a prototype of the *S. cerevisiae* expression vectors used for commercial production of hepatitis B Ag and other clinically useful protein reagents (22). The allergen is expressed as a nonfusion protein at 38 mg/L yeast culture.

Using purified recombinant protein, specific polyclonal antiserum was produced in rabbits and used to confirm the location of the allergen in the salivary gland cells and saliva of *T. protracta*. The antiserum also recognized the corresponding native allergen in *T. protracta* extracts and the recombinant protein was in turn recognized by serum from an allergic individual. Expression and purification of this recombinant allergen provides reagent quantities of protein for future use in serologic testing to identify individuals at risk for hypersensitivity to the bite of this insect. Further studies will determine whether recombinant procalin may also be used to desensitize persons with severe allergy to *T. protracta*.

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